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Trace Elements in Man and Animals

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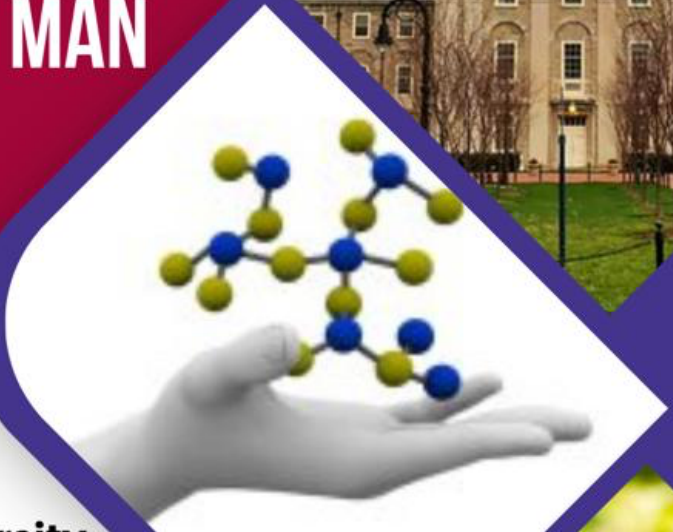

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Keynote Lectures

Prof. Eric Underwood Memorial Lecture

Selenium and selenoproteins in health and disease

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Selenium exerts its essential functions in biology and medicine primarily through selenocysteine-containing proteins. There are 25 genes coding for selenoproteins in humans, which are responsible for these functions. These proteins are involved in a variety of processes but are enriched for oxidoreductase functions. This presentation will discuss the full set of selenoproteins, the selenoproteome, redox properties of these proteins and their changes in response to environmental factors. Additionally, the role of selenium and selenoproteins in aging and age-related diseases will be discussed, along with phenome-wide effects of selenium. The talk will also cover a clinical case of deficiency in selenocysteine machinery.

Zinc in immunosenescence and malnutrition

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Zinc is an essential trace element for the immune system and zinc deficiency compromises the function of all cells of the immune system. However, an excess of zinc have negative effects on the immune system too. Therefore, zinc homeostasis must be delicately regulated for an effective immune response. Zinc deficiency is typical for developing countries due to malnutrition. However, also in industrial countries zinc deficiency is typical in the elderly population and in vegetarians and vegans. However, a mild zinc deficiency is hardly to detect, since a low serum zinc levels is only detected in severe zinc deficiency. Therefore, we established an app-based food-frequency-questionnaire to detect mild zinc deficiency. In the elderly population we have a complex decline of immune function called immunosenescence. However, besides the natural decline of immune function, some typical signs of immunosenescence are due to malnutrition, especially zinc deficiency. Some of the signs of immunosenescence are observed in vegetarians and vegans too and can be restored by zinc supplementation.
Zinc deficiency is accompanied by signs of chronic inflammation. The overexpression of proinflammatory cytokines is due to epigenetic changes in the promoter regions of proinflammatory cytokines during zinc deficiency. Whereas zinc deficiency alters the promoter regions into a more accessible form, a supplementation with zinc reconstructed the promoter to a closed form. However, this is not a complete suppression of proinflammatory cytokines, but a regulation of the threshold of gene activation. This resulted in a higher activity of monocytes under zinc deficient conditions. For T cell immunity the regulation by zinc is the other way around. Zinc supplementation induces a shift towards TH1 immune reactions, which is controlled by regulatory T cells. The production of interferon-γ and interleukin-2 increases with zinc supplementation, which is due to regulation of Zip-8 and CREMa on the molecular level. However, the induction of regulatory T cells limits this effect and is dependent on the zinc concentration and the stimuli used. Here zinc stabilizes the transcription factor FoxP3. A regulation of immune responses in dependence of the zinc concentration could be shown in different models like transplantation, allergy and ageing. Taken together, the immune response is directly regulated by the zinc level and could be manipulated by zinc supplementation in vivo. Therefore, depending on the dose, zinc could be used as immunostimulant for the elderly, vegetarians and vegans, as well as immunosuppressant in autoimmune diseases and allergy.

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6. Trame S., Brüggemann A. and **Rink L.** (2023): Calculating zinc uptake by Zinc-App; J. Trace Elem. Med. Biol. 77:127132
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Invited Talks

Impact of climate change on trace mineral requirements in the first 1000 days

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Among various changing environmental conditions, rising ambient temperatures can potently alter multiple physiologic systems and adversely impact nutritional requirements through impaired bioavailability and increased demand. These effects can impact the entire life course, with notable risks for women (including reproductive outcomes), infants and young children. Acute and chronic inflammation and immunostimulation; increased oxidative stress; altered energy metabolism; and impaired immune function are relevant examples of heat-disrupted physiologic processes that interact with nutrition, including trace minerals. Effects on gastrointestinal integrity provide a direct link to observed disruptions in mineral homeostasis. Epidemiologic data strongly support adverse effects of heat stress on reproductive outcomes, but data related to its intersection with nutritional status are limited. This presentation will highlight examples of physiologic effects of heat stress, through the lens of micronutrient deficiencies and status and interactions with functional pregnancy outcomes, fetal and postnatal growth. Specifically, observations from human trials in pregnant women and animal models of experimental heat exposures on placental development and function and pregnancy outcomes will be reviewed. Effects of heat and air quality as biological variables on anemia and associations with fetal growth provide another example. Lactation and postnatal growth are periods also vulnerable to heat stress, and for which preliminary data will be presented. The presentation will conclude with knowledge gaps related to trace mineral metabolism and interactions with climate and heat exposures, that plausibly underlie clinical observations. Considering current evidence, future research directions and public health agenda will be highlighted.

SELENOP: A dual-role protein in inflammatory bowel disease and colorectal cancer

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Selenoprotein P (SELENOP), a unique selenocysteine-containing protein, plays crucial roles in both redox regulation and selenium transport. Recent research has unveiled its complex involvement in Inflammatory Bowel Disease (IBD) and Colorectal Cancer (CRC). This presentation will review the multifaceted functions of SELENOP in intestinal health and disease.
There is an inverse correlation between serum SELENOP levels and the risk of IBD and CRC. Animal models demonstrate that a reduction in SELENOP exacerbates colitis severity and colitis-associated carcinoma. Paradoxically, complete loss of SELENOP appears protective against tumorigenesis, highlighting the complex nature of oxidative stress in cancer development.
This research provides novel insights into SELENOP's mechanisms of action, including its interaction with key signaling pathways involved in intestinal homeostasis and often dysregulated in CRC. In various experimental models, we, and others, have explored SELENOP's impact on tumor development, growth, and gene expression. Additionally, analysis of human single-cell RNA-sequencing datasets offers valuable information about SELENOP expression patterns in different cell populations during normal function and tumorigenesis.
While SELENOP shows potential in modulating CRC and IBD, we underscore the need for a nuanced understanding of its roles. The protein's involvement in immune cell function, cytokine production, and broader cellular signaling pathways suggests both protective and potentially protumorigenic effects. This research provides a foundation for future investigations into the precise contributions of SELENOP in intestinal disease and its potential as a therapeutic target.

Chemotherapeutic effects of organodiselenides (R-Se-Se-R) in lung cancer cells: Evidence of redox modulation towards reductive stress

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Organodiselenide, a class of organoselenium compounds, is structurally defined as R-Se-Se-R, wherein R is an alkyl or aryl group [1,2]. Organic diselenides are well documented for redox modulatory activity activities in biological systems [2]. For instance, such molecules can behave like an antioxidant by undergoing reduction either through NADPH in a thioredoxin reductase (TrxR) catalysed reaction or through glutathione (GSH) to form selenol (R-Se-H) or selone (R-Se=C) [1-3]. The reduced species in turn can catalyse the reduction of hydrogen peroxide into water molecules by a glutathione peroxidase (GPx)-like catalytic mechanism. Alternatively, organodiselenides can oxidize cellular GSH in futile cycle leading to generation of reactive oxygen species (ROS) and thus can act like pro-oxidants. [1-3]. Such paradoxical behaviours of diselenides make them excellent candidates for dual activities of chemoprevention and chemotherapeutic applications. Of these, the chemotherapeutic effect of organodiselenides is mainly linked to their prooxidant activity [2]. On similar lines, our research group has evaluated several synthetic organo-selenium compounds for chemotherapeutic/radioprotector applications and has identified a potent alkyl diselenide, called 3,3'-diseleno dipropionic acid (DSePA), exhibiting differential activity of radioprotection in normal cells and anticancer effect/radio-sensitization effect in tumor cells [3-5]. The chemotherapeutic effect of DSePA has primarily been studied in cellular and murine xenograft tumor models of lung cancers [3]. The mechanism of the anticancer activity of DSePA studied so far has revealed that it activates the biological mechanism of cell cycle perturbation, unfolded protein response, and apoptosis in lung cancer cells through reductive stress (marked by significant decrease in the basal level of ROS and a concurrent increase in the ratio of GSH/GSSG and NADH/NAD) rather than the commonly presumed concept of oxidative stress [3]. Notably, 2,2'-dipyridyl diselenide (Py_2Se_2), an aryl diselenide also showed similar observation of reductive stress mediated chemotherapeutic effect in the cellular model of lung cancer [6,7]. Together, these findings present significant advancement towards the existing understanding of the redox biology of selenium compounds and their chemotherapeutic applications. The results pertaining to these novel observations and the related mechanisms will be presented during the conference.

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Ionomic profiling of patients with colorectal cancer

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The existing data demonstrate that the patterns of trace element metabolism in cancer strongly depend on the affected tissue, stage of the disease, and other variables. Therefore, the objective of the present study was to assess serum and cancerous tissue trace element and mineral levels in colorectal cancer (CRC) patients, and its relation to disease severity. The level of Ca, Co, Cr, Cu, Fe, Mg, Mn, Mo, Se, V, Zn in blood serum and cancerous tissues (only CRC cases) was assessed by inductively-coupled plasma mass-spectrometry in 90 patients with colorectal cancer and 97 age- and sex-matched controls. The obtained data demonstrate that colorectal patients are characterized by 7%, 21%, 24%, and 19% lower Ca, Fe, Se, and Zn levels when compared to healthy controls. In contrast, serum Co, Cu, and Mg, as well as Cu/Zn ratio exceeded the respective control values by 80%, 11%, 5%, and 50%. Serum Zn ($r = 0.278$; $p = 0.032$) and Cu/Zn ($r = 0.270$; $p = 0.039$) values positively correlated with the respective tumor values. Multiple linear regression analysis demonstrated that tumor size (T) was a significant negative predictor of serum Se levels (0.004), being positively associated with serum Cu/Zn values ($p = 0.009$). In turn, the degree of metastasis to regional lymph nodes (N) was inversely associated with circulating Ca ($p = 0.024$), Co ($p = 0.011$), Mg ($p = 0.009$), Zn ($p = 0.019$), and Mn ($p = 0.001$) levels. The predictive ability of the regression models was significant for the studied elements, and especially Se, Zn, and Cu/Zn ratio, accounting for 24%, 25%, and 48% variability of these parameters. These findings demonstrate that in addition to its association with CRC staging, systemic Cu/Zn ratio may be indicative of Cu and Zn imbalance in cancerous tissue, hypothetically involved in its progression.

Novel developments in the selenoprotein biosynthesis pathway: Genes, biochemistry, and human disease

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Selenium is an essential trace element. In mammals, the essentiality of selenium was attributed to its presence as selenocysteine (Sec) in selenoproteins. The human genome contains 25 genes encoding selenoproteins. Among these are the well-known glutathione peroxidases (GPX), thioredoxin reductases, and iodothyronine deiodinases as well as a number of selenoproteins of which some have not yet a clearly assigned biochemical or physiological function. The canonical pathway of selenoprotein biosynthesis requires several factors, including a $tRNA^{Sec}$, phosphoseryl-tRNA-kinase (PSTK), selenophosphate synthase 2 (SEPHS2), selenocysteine synthase (SEPSECS), a specific elongation factor (EFSEC), and SECIS-binding protein 2 (SECISBP2). The latter protein recognizes the selenocysteine insertion sequence (SECIS) element present in the 3'-untranslated region of selenoprotein mRNAs. Selenium distribution in the body may occur as inorganic selenite or within the plasma protein selenoprotein P (SELENOP). It becomes more and more clear, that cells or organs with a high requirement for selenium express lipoprotein-receptor-related protein LRP8, while other organs may utilize LRP2 or LRP1. Most of this research is grounded on studies in animals, mostly genetically engineered mice.
The increasingly widespread application of exome sequencing has led to the identification of a growing number of human individuals carrying pathogenic variant is selenoprotein genes or genes involved in selenoprotein biosynthesis. In humans, there appear to exist two syndromes of selenoprotein deficiency: one with predominantly thyroid hormone-related symptoms, and one with neurological symptoms. Moreover, GPX4 is an essential enzyme protecting cells, also cancers, from ferroptosis. In addition, deiodinases have been more recently implicated in cancer.
I will summarize human cases of selenoprotein deficiency, compare phenotypes between mice and patients, and present some new findings on genes and genetic variants in the pathway, which in the future may bear relevance for oncology.

Regulatory frameworks for selenoprotein expression

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With the recent CryoEM structure of the “selenosome” culminating many years of biochemical characterization, the core mechanism by which selenocysteine is incorporated into selenoproteins is coming into focus. The current overriding mechanistic question in the field is now about how Sec incorporation is regulated. It is well known that selenoprotein expression is exquisitely and differentially regulated by a myriad of conditions including selenium concentration, tissue type, oxidative stress and iron concentration. While transcriptional regulation is certainly relevant, post-transcriptional mechanisms are likely dominant due to the complexity of Sec incorporation. The only known feature in the Sec incorporation machinery that would allow differential regulation among selenoproteins is the Sec insertion sequence (SECIS), which is strictly required for delivery of Sec to ribosomes. In addition, SECIS elements are found in highly variable 3' UTR contexts. Strikingly, neither SECIS elements nor 3' UTR are conserved across selenoprotein mRNAs, providing clear evidence or regulatory potential. As a model, we studied the selenoprotein that catalyzes phosphoethanolamine synthesis, SELENOI, in part because it has the longest 3' UTR among all selenoprotein mRNAs (~7kb). Using a variety of reporter and native cDNA constructs, we have found strong inhibitory elements in the SELENOI 3' UTR that are differentially utilized. In addition, using CRISPR edited cell lines, we have found that the SELENOI SECIS has a strict requirement for SBP2 versus SECISBP2L. The significance of regulated Sec incorporation into SELENOI is still speculative but very likely to include induction during differentiation of oligodendrocytes, which produce myelin sheath in the CNS.

Halogen Bonding with Selenium and Iodine: Thyroid Hormone Deiodination and Membrane Transport

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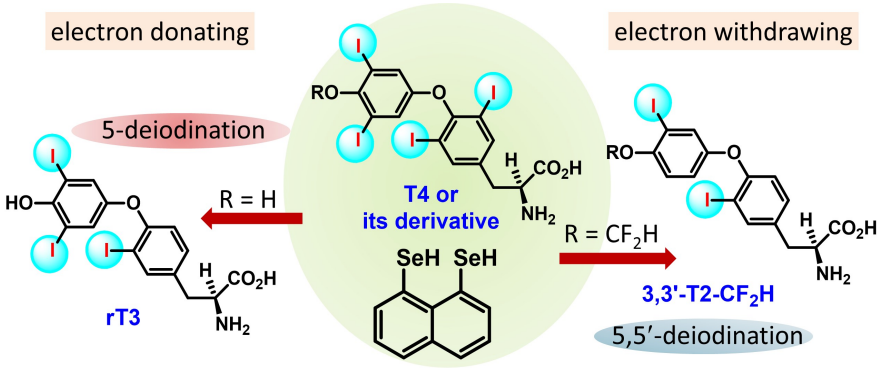
Selenium and iodine, the two essential trace elements, play key roles in many biological processes. While selenium exists in the form of selenocysteine, the 21st amino acid, in many proteins, including glutathione peroxidase (GPx), iodothyronine deiodinase (Dio) and thioredoxin reductase (TrxR), iodine is an integral part of the thyroid hormones, L-thyroxine (T4) and its metabolites. The non-covalent interactions between these two trace elements play essential roles not only in protein conformation but also in enzyme-mediated catalysis.



In this lecture, the chemical mechanism by which the deiodinases and organoselenium compounds selectively activate and inactivate the thyroid hormones will be discussed. The role of iodine atom and halogen bonding in the membrane transport of fluorescent molecules will also be discussed.

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Functional adaptation of mammalian HRG1/SCL48A1 as an intestinal heme transporter

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Heme is an essential cofactor in a multitude of metabolic pathways. Since heme is a hydrophobic and cytotoxic molecule, its transport and trafficking needs to be tightly regulated. We identified the first eukaryotic heme transporter HRG1/SLC48A1 using *Caenorhabditis elegans* as a genetically tractable animal model. The human homolog of HRG1 transports heme from the phagolysosome to the cytosol in macrophages after erythrophagocytosis. Surprisingly, HRG1-KO mice could tolerate heme loading by forming heme crystals called hemozoin (Hz), previously reported only in malarial parasites, within the phagolysosomes of reticuloendothelial in macrophages. Here, we show that HRG1 is a high affinity heme transporter enriched in enterocytes at the tip of the microvillus in the small intestine. HRG1 is regulated by heme and iron, forms homo-multimeric complexes, and its activity is stimulated by acidic pH. Unexpectedly, HRG1 localizes to both, apical plasma membranes and subapical vesicles, in polarized human intestinal organoids and in humanized *C. elegans* that lack all paralogous heme transporters. This dual membrane localization of HRG1 is dictated by non-canonical sorting motifs within its cytoplasmic carboxyl-terminus and specific membrane-trafficking factors. High-speed volumetric live-cell imaging reveals HRG1 is rapidly recycled from the plasma membrane to endolysosomal compartments. Our studies demonstrate that mammalian HRG1 functionally adapted from its ancestral invertebrate homologs to support both heme-iron recycling and heme transport through dual membrane localization.

Role of selenoproteins in stress erythropoiesis

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Steady state erythropoiesis is primarily homeostatic. It produces 2.5 $x10^6$ new erythrocytes per second, which replace the 2.5 x 10^6 senescent erythrocytes that are removed each second. To maintain homeostasis, erythropoiesis is highly regulated. Infection or injury that causes inflammation alters hematopoiesis, skewing production towards myelopoiesis to make effector cells to fight infection and heal wounds. In addition, pro-inflammatory signals increase erythrocyte turnover and sequester iron making it unavailable for hemoglobin synthesis. To compensate for this loss in production, extramedullary stress erythropoiesis is induced. Stress erythropoiesis uses a different strategy than steady state erythropoiesis. Stress erythroid progenitors (SEPs) are derived directly from short-term hematopoietic stem cells. Pro-inflammatory signals drive the proliferation of self-renewing immature SEP populations. Epo signaling in the niche transitions this transient amplifying population to committed erythroid progenitors that lose their stem cell characteristics and differentiate. Previous work from the lab showed that blocking selenoprotein synthesis using a conditional allele of Trsp completely impaired stress erythropoiesis. Loss of selenoproteins affected both SEPs and the macrophage monocyte niche. We have continued these investigations and have focused on the roles of SelenoW and SelenoN in SEPs. Using mutants in these two proteins we have identified defects in the expansion of SEP populations and their commitment to differentiation. Our work shows that prostaglandin E_2 (PGE_2) signaling is a key signal driving the commitment of SEPs from self-renewing immature progenitors to committed erythroid progenitors. PGE_2 mobilizes intracellular Ca^{2+} to induce commitment and SelenoN mutation affects Ca^{2+} signaling and impairs the coordination between proliferation and commitment to differentiation. In contrast, SelenoW appears to affect both the SEPs and the niche.

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Nutritional and molecular approaches to modulate intestinal iron absorption in iron-deficiency and iron-overload-related disorders

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Iron is an essential nutrient for humans. Iron deficiency is the most common cause of anemia globally, resulting in significant morbidity and mortality. Iron is also toxic when in excess, due to its propensity for generating damaging oxygen free radicals. Iron overload is most frequently observed in inherited genetic disorders, including hereditary hemochromatosis and β-thalassemia. Overall body iron levels are dictated by the magnitude of the absorption of enteral iron, since no active, regulated means exists to excrete excess iron. Regulation of intestinal iron absorption is complex, involving local, cell-based and systemic effectors. Both main forms of iron in the human diet, nonheme (or inorganic) iron and heme iron, are absorbed in the proximal small intestine by distinct, but possibly overlapping, mechanisms. Extensive experimentation over the past 20+ years has demonstrated that many mouse models of iron-related disorders faithfully recapitulate many aspects of these conditions in humans. The overall goals of our research program in recent years have focused upon approaches to modulate intestinal iron absorption using mouse models. New iron supplementation approaches have been pursued that may allow lower functional doses of iron to be used, thus decreasing notable negative side effects of excess enteral iron. One such approach sought to identify amino acids that increased expression of the main nonheme iron importer, divalent metal-ion transporter 1 (DMT1), on the apical membrane of duodenal enterocytes. The rationale was based on our previous work in which we demonstrated that certain amino acids could influence expression or activity of intestinal nutrient transporters. We also tested whether DMT1 is a plausible therapeutic target in mouse models of iron-loading disorders (i.e., hereditary hemochromatosis and β-thalassemia), in which we tested the hypothesis that the excess iron absorption in these conditions was mediated by DMT1. Whether *in vivo* silencing of intestinal DMT1 mitigated iron loading in these iron-loading disorders was also considered. Recent work in the lab has also allowed us to develop experiments to consider how dietary nonheme iron and heme iron may differentially influence iron supplementation regimens and whether the different forms of iron lead to differences in iron loading. Collectively, these approaches may provide new outcomes that could allow the development of improved nutritional and genetic therapies to modulate iron absorption in these and other related disease states.

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Zinc supplementation in diabetes and prediabetes. Current knowledge and challenges

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Type-2 diabetes (T2D) is a highly prevalent condition. Worldwide, it affects about one-tenth of the adult population. It is a chronic condition responsible for long term-severe dysfunction of several organs. Besides, it is a heavy burden on the health system. Prediabetes is a glucose homeostasis disorder that precedes T2D, and it is estimated to account for approximately three times the prevalence of T2D. A significant proportion of patients with prediabetes will eventually develop T2D.

Zn plays a role in the secretion and action of insulin. Specifically, Zn participates in insulin processing, storage, and secretion. In addition, Zn has insulin-mimetic properties, participating in insulin signaling cascades in peripheral tissues. Zn may decrease diabetes-related glucotoxicity and lipotoxicity due to its antioxidant, anti-inflammatory, and anti-apoptotic properties. Thus, sound biological bases suggest that Zn could be a co-adjuvant in preventing and managing T2D. There are meta-analysis in the literature on Zn supplementation and its effects on glycemic control and some risk factors in individuals with T2D. The information on prediabetes is very limited. Although all meta-analysis suggest some extent of beneficial effects on glycemic control biomarkers, and/or cardiovascular risk factors, results have been heterogeneous, mainly due to the differences in study designs and parameters used to assess the impact of interventions. Additional factors affecting the outcome are the individuals' characteristics before the intervention, including Zn status, glycemic control medications, and duration and time of intervention since diagnosis of T2D or prediabetes. A closer look at such factors leads to the need for better and improved experimental designs to clarify the impact of Zn supplementation on the course of these conditions.

The trace element-dependent thyroid hormone system - novel deiodinase selenoenzyme inhibitors

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Thyroid hormones (TH) are indispensable regulators of development, growth, energy metabolism and maintenance of the bodily functions required to adequately respond to challenges of environment, disease and survival. Biosynthesis, distribution, metabolism and action of iodinated TH crucially depends on three essential trace elements: iodine, selenium and iron. However, the nutritional supply the of these trace elements is inadequate in many regions of the world. This renders the TH producing thyroid gland and the entire TH system vulnerable to environmental disruptors, especially during early fetal TH-dependent brain development. Activation of the prohormone thyroxine (T4), its inactivation, and the formation of active 3,3',5-triiodo-L-thyronine (T3) are catalyzed by the deiodinase (DIO) selenoenzyme family. DIOs sequentially remove iodide from TH by reductive cleavage of C-I bonds. DIOs, expressed and regulated in cell-, development-, (patho-)physiological and spatio-temporal manner, control local intracellular availability of T3 binding to its receptor. T3 receptors, ligand-dependent transcription factors, modulate expression of T3-dependent genes. DIOs are low abundance integral membrane proteins with their active sites oriented toward cytosol. Enzyme kinetics, reaction mechanisms and substrate preferences are known, while endogenous reductants, isoenzyme-specific inhibitors and full structures remain to be identified, apart from the crystal structure of the mouse Cys-homologue Dio3 catalytic core and the DIO1-specific inhibitor PTU, an antithyroid drug. Using human DIO homogenates as enzyme source, recombinantly expressed in HEK-293 cells, and the non-radioactive Sandell-Kolthoff reaction to detect enzymatically released iodide. we developed and applied a robust and versatile high-throughput screening platform to identify DIO-selective and pan-inhibitors by screening large libraries (50-60K) containing diverse sets of low molecular weight compounds, probes, building blocks for pharmaceuticals, approved drugs and environmentally relevant chemicals. Identified potent DIO-selective inhibitors need further characterization for their potential application as research tools, reference compounds and administration in more complex in vitro organoid or in vivo animal experimental models.

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Antioxidant activity and cytotoxicity of selenium incorporated biologically inspired N-heteroaryl compounds

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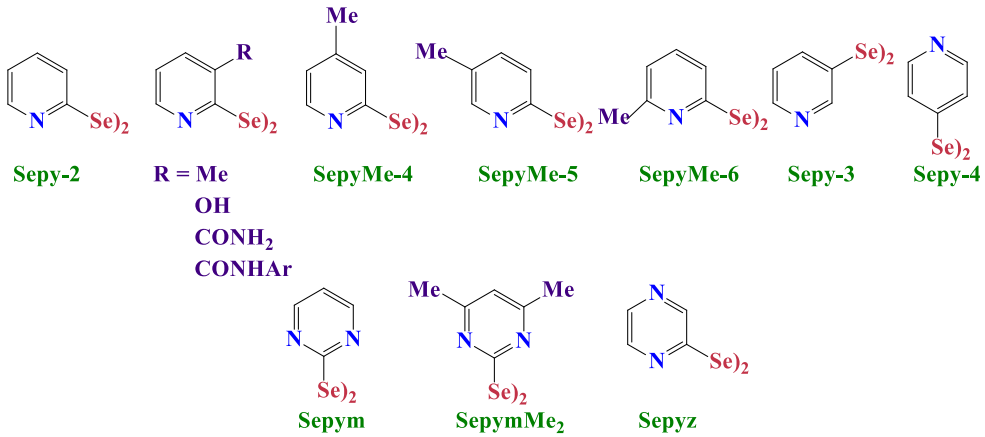
Selenium is an essential trace element for life. It is incorporated in the form of redox-active selenocysteine (Sec) in a limited number of proteins that exhibit a variety of functions like reducing oxidative stress, maintaining redox homeostasis, thyroid hormone metabolism, *etc*. Pyridyl based (*e*.g., 2-pyridinol, 2-nicotinamide, *etc.*) systems constitute a large family of biomolecules that are also responsible for a wide range of biological activities like redox reactions (*e. g.,* NAD ⇌ NADH; $NADP^+$ ⇌ NADPH). Being endogenous in nature, these scaffolds are extensively employed as pharmaceutical motifs in drug design and development as evident from the FDA database which reveals that more than 60% small molecule drugs are composed of N-heterocycles. Accordingly, we designed and synthesised a variety of selenium incorporated pyridyl derivatives with reference to their antioxidant activity (Scheme 1). Their structures and polymorphism have been studied. Antioxidant activity and cytotoxicity of these compounds have also been investigated. The nature of substituents at C-3 position of the pyridyl ring has a profound effect not only on the structure of the molecule but also on the antioxidant activity and cytotoxicity.



Scheme-1

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Probing one- electron transfer reactions in selected trace elements

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Electron transfer is a key process in many biological redox reactions like photosynthesis, respiration, peroxidases etc. Most of these processes are mediated by trace elements like transition metals and chalcogens. Although the overall redox reaction often involves transfer of multiple electrons, it may happen in several steps of single electron transfer processes. Understanding and probing such one electron processes is necessary to optimise the energy channelling in the mimetic redox biology, however monitoring such reactions requires advanced spectroscopic tools. In transition metals due to the availability of stable oxidation states, one-electron transfer can be monitored by steady state techniques, microscopy, NMR XRD etc. But such process involving p-block chalcogen elements like oxygen, sulfur and selenium requires real time measurements due to the involvement of short lived free radical species. Our group has contributed significantly to the study of one-electron transfer reactions in these systems using nanosecond pulse radiolysis facility and identified crucial steps involved in redox processes in enzyme mimcs. Examples include superoxide dismutase activity of copper-curcumin complexes and glutathione peroxidase activity of small organoselenium compounds. Results from some of these studies will be discussed in the lecture.

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Advances in selenium speciation analysis in established and emerging food supplements

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Selenium is a vital trace element crucial for the health and well-being of both humans and animals. It participates in a number of key physiological processes, such as antioxidant defense, immune system support, and thyroid hormone metabolism and, as a result, selenium deficiency can result in serious problems necessitating supplementation. Consequently, there is a demand for selenium-enriched food and feed supplements. Organic selenium compounds are generally more bioavailable compared to inorganic forms so the elaboration of the supplements usually involves fast growing microorganisms able to convert inorganic selenium salts into organic selenospecies. Selenium-enriched yeast, *Saccharomyces cerevisiae*, is the most widely used organic selenium supplement although other yeast varieties are also becoming popular. Additionally, certain lactic acid bacteria such as, *e.g.*,*Bifidobacterium animalis* can also be used; they show significant therapeutical effects and draw significant attention of nutritionists and pharmacologists. At the same time, a large variety of food products (naturally or artificially) enriched in selenium are commercialized. The last but not least, novel supplements based on selenium nanoparticles are becoming popular.

The biological effects of selenium are highly dependent on its specific chemical forms. Inorganic selenium is metabolized by living organisms into a wide array of species. The identification and quantification of them is crucial for understanding selenium's molecular role and metabolism.

The presentation will highlight recent advances in mass spectrometry-based methods for characterizing selenium species in established and emerging food supplements. The approaches involve fractionating biological extracts *via* chromatography, with specific selenium detection using inductively coupled plasma mass spectrometry (ICP MS) and high-resolution Fourier-Transform electrospray mass spectrometry (ESI MS^n) to elucidate the structures of detected selenospecies. A dedicated methodology - Single Particle - ICP MS - used for nanoparticle analysis will be also discussed.

Molecular metallomics: An approach to study trafficking of essential trace elements in pathogenic bacteria

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The concentrations of essential transition metals (e.g., Fe, Co, Ni) in biological systems are regulated by an organism's physiology and biochemistry. Metal-induced gene expression leads to the synthesis of numerous biological ligands at the metabolome level, influencing the uptake, trafficking, accumulation, and excretion of trace elements. Therefore, understanding metal speciation is crucial for comprehending the role and fate of metals in a biological system at the molecular level. Linking this knowledge with the organism's genome is a key challenge in the emerging field of metallomics [1].

Generally associated with organic molecules, metals are usually present in low concentrations (nM to µM range) and occur in a wide range of chemical forms with various properties (covalent or noncovalent species and diverse molecular weights). HPLC combined with ICP MS and electrospray MS/MS detection has provided most of the current data on the chemical forms of essential elements in bacterial extracts.

The lecture will discuss the state-of-the-art and limitations of mass spectrometry-based analytical methods for the non-targeted analysis of essential metal complexes in biology. The importance of linking analytical data with gene expression related to enzyme production will also be highlighted [2].

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Selenium, sex, stress, brains and energy

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Selenium (Se) is an essential trace element whose functions are largely attributed to its incorporation into selenoproteins in the form of selenocysteine. Se exhibits a narrow range for beneficial health effects, whereas Se deficiency or excess are both associated with detrimental health consequences. Further complicating the picture, Se metabolism and selenoprotein expression and regulation exhibit sexual dimorphism, with patterns of regulation frequently diverging between rodents and humans. This presentation will provide an overview of Se and sex, previously addressed in the primary literature and reviews, and highlight some of the more pressing unresolved questions. New data will also be presented from two ongoing studies in our laboratory: 1) the interactions between Se, glucocorticoid treatment, sex, weight gain and energy metabolism in mice, and 2) the relationship between midlife dietary Se and late life cognitive decline or dementia, and cerebral infarcts observed at autopsy, assessed in a large cohort of Japanese-American men in Hawaii between 1965 and 2012.

Role of astrocytic selenoprotein T in synaptic plasticity of the visual cortex

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Selenoprotein T (SELENOT) is a selenocysteine-containing protein located in endoplasmic reticulum, and is implicated for regulating calcium homeostasis. We have previously shown that SELENOT expression is increased in patients and models of Parkinson's disease, and promotes G1-to-S cell cycle transition in SK-N-SH neuroblastoma cells, linking a protective role of SELENOT to neural proliferation. Synaptic plasticity is the activity-dependent change in the strength of neuronal connections. Its dysfunction is associated with a number of brain disorders including autism, depression and amblyopia. Astrocytes support and provide nutrition for neurons but are also involved in the regulation of synaptic plasticity in central nervous system. Herein, we aimed to understand the role of astrocytic SELENOT in synaptic plasticity of the visual cortex by using conditional knockout mice and monocular eye deprivation or enucleation model. We assessed expression changes of SELENOT and the synaptic plasticity marker Arc in the visual cortex of the modeled mice, measured the stereoscopic vision by visual cliff behavior, functional integrity of the visual pathway by flash visual evoked potential, excitation/inhibition balance by electrophysiology, and explored the underlying mechanisms. Overall, we demonstrate that SELENOT defect in the astrocytes impairs neuronal synaptic plasticity in the cortex.

A zinc sensing receptor in epithelial function and disease

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Zinc ions serve as signaling molecules regulating many cellular functions via modulation of membrane receptors and transporters, or by regulation of kinases and phosphatases activity. However, a unique selective target for Zn2+ is the zinc sensing receptor ZnR/GPR39, which is a G□q-coupled receptor. As such, ZnR/GPR39 serves as a mediator between changes in extracellular Zn2+ concentration and cellular Ca2+ signalling that activates downstream kinases linked to epithelial cell proliferation and survival. Notably, ZnR/GPR39 regulates keratinocyte cell proliferation and wound healing, a process associated with zinc. ZnR/GPR39 also enhances colon epithelial barrier function by enhancing cell proliferation and tight junction expression and thereby protects the epithelial wall during dextran sodium sulfate (DSS) model of colitis. Our recent results also show a role for ZnR/GPR39 in hepatocyte function, where ZnR/GPR39 knockout mice exhibit decreased junctional protein expression leading to increased liver inflammation and steatosis. Altogether, our data suggest that ZnR/GPR39 is a unique signaling target for therapeutically protecting epithelial barrier function during ulcerative or inflammatory syndromes.

Elemental Escorts: Secret lives of trace element transporters

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In the iron overload disorders hereditary hemochromatosis and thalassemia major, iron appears in blood plasma as non-transferrin-bound iron (NTBI), which is the major contributor of iron loading of tissues. We have previously shown using knockout mice that the metal-ion transporter SLC39A14 (ZIP14) is required for iron uptake and accumulation by the liver (hepatocytes) and pancreas (acinar cells) in mouse models of iron overload. In recent studies using cardiomyocyte-specific *Slc39a14* knockout mice, we have found that SLC39A14 is also the primary driver of iron uptake by cardiomyocytes in iron-loaded mice. Given the central role of ZIP14 in tissue iron loading, we are currently exploring antisense oligonucleotide (ASO) technologies as a potential add-on therapy to iron chelation for the treatment of iron overload. Our studies show that ASOs targeting *Slc39a14* in combination with the oral iron chelator deferiprone are more effective than iron chelation alone at decreasing liver iron levels and body iron burden in hemochromatotic mice. Interestingly, despite its role in mediating pathologic NTBI uptake, SLC39A14 functions physiologically primarily as a manganese transporter, as confirmed by studies of *Slc39a14* knockout mice and humans with loss-of-function mutations in *SLC39A14*, who present with brain manganese accumulation and early onset parkinsonism. More recently, we have found that brain manganese (and likely NTBI) uptake is mediated by ZIP8 (SLC39A8), a close homologue of ZIP14. Together, these findings reveal novel links between iron and manganese transport, highlighting how physiologic context affects substrate selectivity and transport.

Targeting oxygen homeostasis to treat diseases of copper metabolism

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Copper and oxygen are essential elements in biological systems, where their roles are tightly regulated. Copper is critical for key enzymatic processes, including mitochondrial respiration, connective tissue formation, and neurotransmitter synthesis. However, when copper homeostasis is disrupted, it can lead to severe diseases such as Menkes disease, marked by copper deficiency, and Wilson disease, characterized by copper toxicity. More recently, a novel copper-dependent cell death mechanism known as cuproptosis has been discovered. This pathway is activated by the accumulation of excess copper in mitochondria, which results in lipoylated protein aggregation and subsequent cell death.

This study aimed to identify new suppressors of cuproptosis by conducting a genome-wide CRISPR knockout screen in a murine cell line lacking key copper resistance genes, including the copper exporter ATP7A and the metallothionein genes, MTI and MTII. The screen revealed that mutations in the von Hippel Lindau (VHL) gene suppressed cuproptosis by upregulating an alternative copper exporter, ATP7B. Consistent with the known role of VHL as a suppressor of hypoxic signaling, exposure to either a low oxygen atmosphere or treatment with hypoxia mimetic drugs also conferred copper tolerance in CuS cells. Additionally, the hypoxia-mimetic drug Roxadustat reduced liver pathology in an Atp7b mutant mouse model of Wilson disease. This research reveals new connections between copper and oxygen regulation and highlights the therapeutic potential of hypoxia-mimetic drugs for treating copper-related disorders.

Hepatic copper overload impairs selenium homeostasis

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Selenium homeostasis depends on hepatic biosynthesis of selenoprotein P (SELENOP) and SELENOP-dependent transport from the liver to target organs such as the brain. In addition to selenium, the liver also regulates copper homeostasis through biosynthesis and excretion of copper-containing ceruloplasmin. Selenium and copper metabolism are linked, and an inverse correlation with increasing copper and decreasing selenium levels is observed in blood during aging and inflammation. Based on this, we hypothesize a direct interference of copper with SELENOP excretion.
We used the human liver-derived cancer cell line HepG2 and primary murine hepatocytes as model to analyze intracellular trace element concentrations, and intra- and extracellular levels of SELENOP in response to copper and selenium treatment. We show that copper treatment increased intracellular selenium and decreased extracellular SELENOP levels. The same effect was supported by an untargeted secretome approach in which SELENOP was particularly downregulated by copper. The reduction of extracellular SELENOP was accompanied by intracellular accumulation of SELENOP. However, a knockout of SELENOP in HepG2 cells abolished the copper-dependent accumulation of selenium indicating specificity of copper for SELENOP.
Accumulation of copper in liver is a characteristic of Wilson's disease. Accordingly, SELENOP levels were relatively low in serum of patients with Wilson's disease.
Mechanistically, treating cells with drugs targeting protein transport in the ER and Golgi mimicked some of the effects observed, indicating a disrupting effect of copper on intracellular SELENOP transport. This appears to affect not only newly synthesized SELENOP but also SELENOP taken up from the medium.
Our data suggest that modulation of hepatic copper levels determine SELENOP release and may affect selenium transport to peripheral tissues such as the brain.

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Copper biology and medicine

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Copper is an essential micronutrient required for the growth and development of all aerobic organisms. Copper is a catalytic cofactor for many critical enzymes, such as cytochrome c oxidase, the primary respiratory enzyme required for mitochondrial energy generation. Mutations that cause systemic or subcellular copper deficiency give rise to fatal infantile disorders, including Menkes disease and a subset of mitochondrial disorders. Despite decades of work, there are currently no approved treatments for these lethal disorders. This is due to our limited understanding of the pathways by which copper is delivered to mitochondrial cytochrome c oxidase. Recognizing that the mitochondrial copper delivery pathway is conserved from yeast to humans, we utilized yeast genetic screens to discover novel members and regulators of this pathway. Using the copper-dependent growth phenotype of one of our newly discovered yeast mutants of this pathway, we designed a targeted screen for copper-transporting drugs. Through this screen, we discovered elesclomol as a potent copper ionophore that transports copper to mitochondria and restores cytochrome c oxidase function in yeast, zebrafish, and mice with genetic defects in copper acquisition. Inspired by our studies, the Spanish Agency of Medicines and Health Products recently approved the exceptional use of elesclomol-copper in infants with Menkes disease, where the initial results are promising. Our work illustrates how fundamental discoveries in yeast can be translated into the development of human therapeutics.

BTBD9 attenuates manganese-induced oxidative stress and neurotoxicity

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Manganese (Mn) is an essential mineral, but excess exposure can cause dopaminergic neurotoxicity. Restless legs syndrome (RLS) is a common neurological disorder, but the etiology and pathology remain largely unknown. The purpose of this study was to identify the role of Mn in the regulation of an RLS genetic risk factor BTBD9, characterize the function of BTBD9 in Mn-induced oxidative stress and dopaminergic neuronal dysfunction. We found that human subjects with high blood Mn levels were associated with decreased BTBD9 mRNA levels, when compared with subjects with low blood Mn levels. In A549 cells, Mn exposure decreased BTBD9 protein levels. In Caenorhabditis elegans, loss of hpo-9 (BTBD9 homolog) resulted in more susceptibility to Mn-induced oxidative stress and mitochondrial dysfunction, as well as decreased dopamine levels and alternations of dopaminergic neuronal morphology and behavior. Overexpression of hpo-9 in mutant animals restored these defects and the protection was eliminated by mutation of the forkhead box O (FOXO). Our results suggest that elevated Mn exposure might be an environmental risk factor for RLS. Furthermore, BTBD9 functions to alleviate Mn-induced oxidative stress and neurotoxicity.

PHD2-HIF-SLC30A10 define a mammalian manganese sensing and response pathway

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This essential metal manganese is toxic at elevated levels and induces parkinsonism. Unlike other metals, mammalian homeostatic responses to manganese were unclear until recently, which hindered therapeutic progress. This presentation will focus on recent work characterizing the first human-relevant manganese sensing and homeostatic pathway. Presented data will show that: (1) elevated manganese outcompetes a catalytic iron atom of PHD2 enzyme, which normally degrades HIF transcription factors, to inhibit the enzyme; (2) manganese-induced PHD2 inhibition induces HIF-mediated transcription, which directly upregulates expression of the manganese exporter SLC30A10; (3) SLC30A10 upregulation provides a pathway to reduce cellular and organismal manganese levels; and (4) pharmacological manipulation of the HIF cascade provides a therapeutic strategy for managing manganese-induced parkinsonism.

The importance of selenium for understanding symptoms of fatigue

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Fatigue is a poorly defined negative health condition with or without psychological distress, poor mental health, poor energy levels, low vigor and rapid exhaustion, as well as additional physical and mental discomfort and low quality of life. It is experienced by patients in conjunction with various noxae, often in response to malignancies, pregnancy, infections, trauma or other illnesses with a strong inflammatory component. Symptoms vary widely, ranging from transient malaise to very severe forms such as chronic fatigue syndrome (CFS), where patients may become constantly bedridden, partly unable to actively communicate with friends and family. Given the overlapping symptoms with severe hypothyroidism, we speculated that thyroid hormone metabolism might be disturbed in severe forms of fatigue.
To test this hypothesis, we conducted three complementary studies aimed at characterizing Se status in patients with Hashimoto's thyroiditis, CFS and severe burns as a model for the response to a severe inflammatory state. Pseudonymized serum samples were obtained and analyzed for total Se and relevant biomarkers of Se metabolism. The results showed a particularly increased prevalence of autoantibodies to selenoprotein P (SELENOP-aAb) in the patients, and a newly developing autoimmunity to SELENOP in response to burn injury. In vitro experiments suggested an antagonistic effect of SELENOP-aAb on Se transport into target cells, and serum analyses confirmed this assumption by detecting impaired expression of Se-dependent GPX3 activity, increased oxidative stress and low conversion of thyroid hormone to the active metabolite T3 in patients positive for SELENOP-aAb. We conclude that some patients with symptoms of fatigue suffer from an acquired autoimmunity-dependent impairment of Se transport into target cells, causing insufficient local thyroid hormone activation in tissues such as brain and muscle, possibly explaining the cardinal signs observed in CFS, such as brain fog and post-exertional malaise.

Fe-S cluster biogenesis and regulation regulates persistence of *M. tuberculosis*

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Iron-Sulfur (Fe-S) clusters are ubiquitous protein co-factors assembled by the complex multi-protein systems, SUF and ISC, in most prokaryotes. *M. tuberculosis* (*Mtb*) encodes a complete SUF system, the depletion of which was bactericidal. The ISC operon of *Mtb* is truncated to a single gene *iscS* (cysteine desulfurase), whose function remains uncertain. We find that *MtbΔiscS* cannot maintain redox balance, carbon metabolism, and oxidative phosphorylation. *MtbΔiscS* exhibited defective survival under oxidative stress, antibiotics, and hypoxia. Transcriptomic analysis indicates that IscS is important for expressing regulons of DosR and Fe-S cluster-dependent transcription factors, WhiB3 and SufR. Unlike wild-type *Mtb*, *MtbΔiscS* could not enter a stable persistent state, continued replicating in mice, and showed hypervirulence. The *suf* operon was overexpressed in *MtbΔiscS* inside murine lungs and reducing SUF expression abrogated hypervirulence. Altogether, *Mtb* re-designed the ISC system to “fine-tune” the expression of SUF machinery for establishing persistence without causing detrimental disease in the host.

Selenium and bacteria: one element to fight them all

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PURPOSE: It is estimated that 7.7 million people died in 2019 due to the infections caused by one of 33 different bacteria, with 5 pathogens (*S. aureus*, *E. coli*, *S. pneumoniae*, *K. pneumoniae*, and *P. aeruginosa*) each being involved in more than 500,000 deaths. Among all deaths, 4.95 million were associated with antimicrobial resistances (AMR). Apart from the main cause of these AMR, which is the misuse of antibacterial drugs, biofilms are part of the arsenal that bacteria possess to avoid the action of the antibiotics. These biofilms consist in complexes of microorganisms that adhere to surfaces and secrete protective extracellular matrices. Among all the efforts to fight bacteria and resistances, the use of selenium (Se) containing compounds is emerging as a promising approach, although the literature to date is scarce. Herein, we report the design of a novel library of 15 Se-containing derivatives and their pre-clinical development, including the assessment of their antibacterial effects towards 8 bacteria species, anti-biofilm formation and removal, and DNA gyrase activity inhibition.

METHODS: The antibacterial effects were determined by using the broth dilution test in four Gram positive (*E. coli*, *K. pneumoniae*, *C. freundii*, and *P. aeruginosa*) and three Gram negative (*S. faecalis*, *S. epidermidis*, *B. sphaericus* and *S. aureus*) bacteria species. Anti-biofilm formation and biofilm removal activities of the hit compound (C13) were evaluated in microplate-based assays with no fluid shear in static conditions. The DNA gyrase activity inhibition of C13 was determined by using the TopoGen Gyrase assay kit (TG1003).

RESULTS: The hit compound C13 presents minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values ranging from 0.1 to 12.5 μg/mL. The C13 compound both inhibits biofilm formation (68.5%) and removes biofilm mass (62.7%) at twice its MIC in *P. aeruginosa*. Finally, this Se-derivative inhibits to a greater extent the DNA gyrase activity when compared with a commercial antibiotic with well-known DNA gyrase inhibition at the same concentration.

CONCLUSIONS: The new Se-derivative C13 has demonstrated outstanding antibacterial effects and biofilm inhibition and removal properties. Therefore, it could be considered a promising candidate to be further developed.

Nanotechnology: A recent tool to improve trace mineral bioavailability in farm animals

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Nanotechnology, the manipulation of materials at the nano scale (1 to 100 nanometers), represents a potential advancement in various fields, including animal nutrition. The nanoparticles possess unique physical and chemical properties due to their small size and increased surface area, can significantly improve the bioavailability of the mineral supplementation of farm animals. Trace minerals such as zinc, selenium, manganese, copper, iron and cobalt can be synthesized as nanoparticles, which enhance the physiological processes, including metabolism, immune function and overall health. The major advantage of nano sized trace minerals is the greater attachment with absorptive surfaces, which enhances their bioavailability, allowing for more efficient absorption and utilization within the animals body. This can lead to a reduction in the total amount of trace minerals needed, thereby minimizing the risk of excessive accumulation, excretion and pollution. Changes in the properties of nano minerals lead to improved antioxidant, antimicrobial and other health improvements in animals. It has recently reported that nano minerals can act as bioactives for improving sperm motility in cryopreserved semen and improves fertility. Moreover, certain nano trace minerals have been found to possess antimicrobial properties and these nanoparticles can effectively combat a range of pathogens, including Multidrug-resistant bacteria. Combination of nano minerals and phytochemicals possess synergistic antimicrobial activity, which contributes to improved intestinal health and a reduction in antimicrobial resistance (AMR), which is essential in maintaining the well-being of farm animals and their performance. Several recent studies suggest that mastitis, piglet anemia and other mineral deficiencies can be prevented by nano mineral supplementation. Overall, the application of nanotechnology in the form of nano trace minerals offers a promising approach to advancing farm animal nutrition. By improving nutrient utilization, this technology represents a significant step forward in sustainable and efficient farm animal management.

Keywords: Nanotechnology, Trace minerals, Bioavailability, AMR, Farm Animals

Functional crosstalk between selenoproteins TXNRD1 and GPX4

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Selenium is an essential trace element for mammals and carries out most of its biological effects through activities of selenoproteins. Recent findings suggest that the functions of selenoproteins glutathione peroxidase 4 (GPX4) and thioredoxin reductase 1 (TXNRD1) display a significant crosstalk in cells. The GPX4-altering ferroptosis-inducing drugs RSL3 and ML162 are direct inhibitors of TXNRD1, and the TXNRD1 substrate TRP14 (also named TXNDC17) is the rate limiting reductase reducing cystine into two molecules of cysteine, used for synthesis of GSH. Interestingly, if TRP14 is impaired, cysteine synthesis from methionine through the transsulfuration pathway is typically upregulated, resulting in an increased antioxidant defense capacity. The implications of these observations will be presented and discussed.

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Zinc status biomarkers: a long quest for the ideal candidate

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Sub-optimal zinc status is thought to be prevalent in about 30% of the global population, but there is a pressing need for sensitive, specific and practical biomarkers to confirm such estimates and identify individuals at risk. This search has been ongoing since the earliest description of human zinc deficiency signs and symptoms, and a plethora of candidate biomarkers has been proposed over 60+ years. Plasma zinc has been used extensively to indicate status at a population level, but it is unsuited to the evaluation of individual status and suitable biomarkers have remained elusive.

This presentation will highlight some of the more promising potential biomarkers and will introduce novel candidates from a large human intervention study completed at the Rowett Institute. These biomarkers proved to be considerably more sensitive to controlled zinc depletion than plasma zinc and are practical for analysis in a basic clinical setting. However, we conclude that specificity remains challenging and that a combination of biomarkers is probably required to differentiate between low zinc status and other pathophysiological conditions.

The role of SELENOF in cancer

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SELENOF is an understudied selenoprotein thought to play a role in certain cancers. Our lab has investigated its role in breast cancer and showed that SELENOF is likely a tumor suppressor. New data supporting this notion shows that Selenof knockout (KO) mice display a significant increase in tumor incidence compared to control mice in a model of chemical carcinogenesis induced by 7,12-dimethylbenz(a)anthracene (DMBA), a potent carcinogen. The majority of tumors arising from DMBA treatment were skin lesions and were confirmed by a pathologist to be mostly cutaneous squamous cell carcinomas (SCC) with a few benign papillomas. Remarkably, skin tumors were 6-fold more frequent in the Selenof KO mice compared to the wild type mice. This data indicates that SELENOF is required for protection of the epidermis against DMBA-induced cutaneous SCC. Of note, ultraviolet (UV) radiation and DMBA both cause DNA damage that is repaired by nucleotide excision repair. When cultured normal human epidermal keratinocytes were exposed to UV radiation SELENOF protein levels increased, suggesting that SELENOF is part of the UV response in keratinocytes. To determine whether SELENOF levels are changing in skin cancer, a tissue microarray of human skin tumors (basal cell carcinoma, squamous cell carcinoma and melanoma) were immune-stained for SELENOF. We find significant reduction in SELENOF levels across all three types of skin cancers compared to adjacent normal tissue. Lastly, selenium is used to treat arsenic poisoning and selenium and arsenic have an antagonistic relationship. Arsenic is a co-carcinogen for skin carcinogenesis and cooperates with UV to promote skin cancers. We also find that arsenic is reducing SELENOF levels in keratinocytes. Based on our in vitro data, mouse data and patient samples, we conclude that SELENOF plays a critical role in skin cancer etiology that warrants further investigation.

Selenium, selenoproteins, and prostate cancer mortality

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Prostate cancer remains a significant health challenge, with 35,250 men dying from it in the US alone. Ethnicity, along with social, environmental, and genetic factors, play a significant role in determining the risk of having and dying from prostate cancer. In the US, African American men are 1.7 times more likely to be diagnosed with prostate cancer and 2.1 times more likely to die of the disease, compared to Caucasians. While there was enthusiasm for using selenium supplementation for prostate cancer prevention, clinical trials have not yielded promising results. Our interest in the 15 kDa selenium-containing protein SELENOF was stimulated by the observation of a statistically significant association between *SELENOF* genetic variations, levels of selenium in the blood, and mortality rates in prostate cancer patients. Since selenoproteins such as SELENOF are regulated at the level of translation due to the need to recognize in-frame UGA stop codons as the codon for selenocysteine, we investigated whether SELENOF levels were different in prostate cancer using tissue microarrays (TMA). Staining TMA slides with anti-SELENOF antibodies revealed that SELENOF was reduced in most prostate cancers compared to adjacent benign regions of the same tissue. Dramatic loss of SELENOF in prostate cancer has now been observed in 3 different prostate cancer TMAs. Using over-expression, knock-down and pull-down assays in cultured prostate cells, we have identified the eIF4a3 translation factor - an RNA binding protein that inhibits the translation of another selenoprotein, GPX1 under low selenium conditions - as a negative regulator of SELENOF translation and being over-expressed in prostate cancer. eIF4a3 binds to the 3'- untranslated region, which is required for the insertion of selenocysteine and possessing polymorphisms that are significantly more frequent among African Americans. These data contribute to developing treatments aimed at improving outcomes and addressing health disparities in prostate cancer.

Sodium selenite-induced cell death is mediated by copper and partially resembles the ferroptosis pathway

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Ferroptosis, a form of regulated cell death driven by lipid peroxidation and typically linked to iron metabolism, has emerged as a key mechanism in various pathologies. This study investigates the mechanisms underlying sodium selenite-induced cytotoxicity in HT22 cells and examines its resemblance to ferroptosis. HT22 cells were treated with sodium selenite and subjected to protective interventions, including ferroptosis inhibitors (ferrostatins), lipoxygenase (LOX) inhibitors, antioxidants, and free radical scavengers. Cell viability, reactive oxygen species (ROS) production, and glutathione (GSH) depletion were measured. Additionally, we examined the role of metal ions by using iron and copper chelators and employed two-photon imaging with a copper ratiometric probe to visualize intracellular copper dynamics.

Our results demonstrate that sodium selenite induces cell death in a dose- and time-dependent manner, with 1 mM selenite completely killing cells within one hour while depleting GSH levels. Consistent with ferroptotic mechanisms, selenite-induced cell death was mitigated by LOX inhibitors, ferrostatins, and hydroxyl radical scavengers. Surprisingly, copper chelators – rather than iron chelators – rescued cells from selenite toxicity in a dose-dependent manner. Two-photon imaging confirmed copper release in selenite-treated cells, which was reduced by copper chelation. Interestingly, copper chelators also protected against cystine deprivation-induced cell death, an established model of ferroptosis. Furthermore, inhibitors of the cystine/glutamate transporter xCT provided protection against selenite-induced death, though selenite toxicity appeared independent of both cysteine and glutamate – both cysteine supplementation and glutamate receptor blockers did not produce any effect suggesting that selenite might enter cells via xCT.

Our findings suggest that sodium selenite induces a copper-mediated cell death pathway that shares key features with ferroptosis. Additionally, cystine deprivation-induced ferroptosis may also involve copper, highlighting a potential copper-dependent mechanism in ferroptotic cell death, traditionally considered iron-dependent. This expands our understanding of ferroptosis and suggests new avenues for therapeutic intervention.

Integration of cellular metabolism to intestinal iron absorption

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Iron, an essential micronutrient, plays a role in erythropoiesis and various cellular processes, including mitochondrial respiration. Cellular and systemic levels are tightly regulated by the hepcidin-ferroportin (FPN) axis, where FPN acts as the sole iron exporter, regulated by hepcidin levels. Dysregulation of this axis underlies disorders such as iron-deficiency anemia and hereditary hemochromatosis. While the integral role of iron in cell metabolism is established, the reciprocal impact remains unclear. Our study demonstrates that cellular histidine is a key regulator of FPN-mediated iron export. We demonstrate that histidine levels modulate FPN function, with excess histidine enhancing iron export, and a decrease in histidine attenuating iron export. Interestingly, during heightened iron absorption, intestinal histidine levels rise, suggesting an integrative mechanism for proper iron export. Mechanistically, we demonstrate the role of histidine in the methionine cycle and its role in FPN methylation and FPN-mediated iron export. The intricate interplay between histidine metabolism and iron homeostasis offers novel insights into therapeutic interventions for iron-related disorders.

Targeting NLRP3 to alleviate iron supplements-induced adverse effects in IBD

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One serious extra-intestinal manifestation of inflammatory bowel disease (IBD) is iron deficiency anemia (IDA). The first choice for IDA therapy is oral iron supplementation; yet the rhetoric that IDA can be 'treated' with iron is far from accurate, given the high incidence of severe side effects. We herein investigated the extent to which NLRP3 (NOD-like receptor 3) inflammasome could influence iron-induced adverse effects in IBD. Colonic inflammation was induced in wild-type (WT) and NLRP3-deficient (*Nlrp3*KO) mice via a chemical colitogen, dextran sodium sulfate (DSS) in drinking water for 7 days and, during recovery phase, mice were administered oral $FeSO_4$ (Fe^{2+}) for 3 days. Intriguingly, Fe^{2+} augmented colonic inflammation in WT, but not in *Nlrp3*KO mice. Histological analysis revealed that Fe^{2+}+DSS treated WT colitic mice displayed more inflammation than *Nlrp3*KO mice. Interestingly, oral administration of β-hydroxybutyrate (BHB, an inhibitor of NLRP3) significantly reduced the Fe^{2+} induced inflammation in colitic WT mice. Next, to evaluate the role of endogenous iron in colitis, we used homeostatic iron regulator protein (HFE)-deficient mice (*HfeKO*), which were noted to be highly susceptible to IL-10R neutralization-induced colitis compared to WT mice. Intriguingly, deficiency of NLRP3 in *Hfe*KO mice *(Hfe/Nlrp3-DKO)* significantly protected them from IL-10R neutralization-induced colitis. In conclusion, our study demonstrated that excess iron could exacerbate inflammation during IBD and that BHB could be used as a therapeutic to mitigate iron-induced inflammation in IBD.

The interplay between dietary selenium deficiency, gut microbiota, and diabetes in mouse aging

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We have previously demonstrated that dietary selenium (Se) deficiency promotes type-2 diabetes-like symptoms in telomere-humanized mice aged 18-24 months and wild-type mice aged 5-8 months. Mice fed diets containing ≤0.10 mg Se/kg display impaired glucose tolerance and insulin sensitivity, suggesting increased susceptibility to type 2 diabetes by suboptimal Se status at levels ≤23% of nutritional needs. The fact that c.a. 25% of sequenced bacteria express selenoproteins suggests competition between gut microbiota and the host for Se, especially when this element is insufficiently available. Here, we sequenced bacterial 16S ribosomal RNA libraries prepared from feces of male and female mice aged 18 and 24 months. Results from principal coordination analysis demonstrated that taxonomic composition, the abundance of selective genus of gut microbiota, and ratios of *Firmicutes*/*Bacteroidetes* were changed by dietary Se deficiency and age in both sexes. However, dietary Se deficiency and age increased the abundance of the phyla of *Verrucomicrobia* in a sex-dependent manner. To explore whether and how reshaped gut microbiota linked dietary Se deficiency to type 2 diabetes, we tested a *Verrucomicrobia* bacterium at the species level in mature and middle-aged male mice and found that oral gavage of such species alleviated dietary Se deficiency-induced glucose intolerance and insulin resistance under an antibiotics-pretreated or a conventional condition. The protection was associated with improved intestinal mucosal barrier function, the thickening of the mucosal layer, reduced endotoxemia, and decreased mRNA expression of selected pro-inflammatory cytokines. Altogether, these results suggest that dietary Se deficiency and age reshape gut microbiota and the changes are linked to the associated type 2 diabetes-like and metabolic disorder symptoms.

Interactions of a selenium analog of glutathione with biomolecules

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Selenoglutathione (GSeH) is a water-soluble tripeptide, in which the sulfur atom of biologically important reductant glutathione (GSH) is replaced by a selenium atom. This selenium analogue of glutathione is easily oxidized even by oxygen dissolved in the solvent, so GSeH is usually obtained as the stable oxidized diselenide form, i.e., GSeSeG. GSeH/GSeSeG is known to be useful for oxidative folding of proteins and detoxification of organic mercury [1]. The purpose of this study is to establish the highly efficient synthetic protocol and apply the obtained GSeSeG to various biochemical reactions [2]. Applying the Fmoc strategy of a liquid-phase peptide synthesis (LPPS) method, GSeSeG was successfully obtained in an overall yield of 90 % starting from a selenocysteine derivative Fmoc-Sec(PMB)-OH. The obtained GSeSeG was subsequently reacted with H_2O_2, methylglyoxal (MG), or 1-chloro-2,4-dinitrobenzene (CDNB). These cell-free assays revealed that GSeSeG has a glutathione peroxidase (GPx)-like anti-oxidative stress activity, as well as glyoxalase 1 (GLO1)-like anti-glycative stress activity, in the presence of glutathione reductase (GR) and NADPH, which can activate GSeSeG to GSeH effectively. It was also found that GSeSeG possesses glutathione *S*-transferase (GST)-like detoxification activity. The cell-cultured assays using HeLa cells also demonstrated that GSeSeG shows low toxicity and high anti-stress activity against H_2O_2 and MG. According to these results, we suggest that the unique reactivity of GSeSeG can be applied in the fields of applied biology and medicine.

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Methylation metabolism of chalcogen elements

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Chalcogen (the group 16) elements have unique physicochemical and biological properties. Sulfur and selenium are essential elements of animals, but tellurium is a non-essential one. Selenium is excreted into urine as a selenosugar and trimethylselenonium ion (TMSe). It is reported that the urinary selenosugar is a physical metabolite of selenium. When selenium is ingested beyond the metabolic capacity, TMSe is produced to detoxify the surplus amounts of selenium. Contrary, tellurium is excreted into urine as an only trimethyltelluronium ion (TMTe), and no tellurosugars have not been detected in urine. Sulfur is not a trace element, and is multiply metabolized and utilized in a body. However, no thiosugar have not been also identified in urine. We intended to clarify the molecular mechanisms underlying the methylation of selenium, tellurium and sulfur. In this study, we evaluated the methylation of the chalcogen elements by inorganic (inductively coupled plasma) and organic (electrospray ionization) mass spectrometry. A chemiluminescence-based enzyme assay was also used. We observed that two methyltransferases, i.e., thiopurine *S*-methyltransferase (TPMT) and indolethylamine *N*-methyltransferase (INMT) cooperatively acted to produce the methylated metabolites of selenium, tellurium and sulfur. Namely, TPMT drove the first methylation, and INMT drove the second and third methylation. In addition, supersulfides were methylated with TPMT. According to the results, we concluded that surplus amounts of essential chalcogen and even a trace amount of non-essential chalcogen were metabolized by the common methylation pathway.

Biochanin-A and phloretin mitigate arsenic and chromium-induced renal damage via modulating oxidative stress and SIRT1/NRF2/HO-1/NQO1 pathway

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It has been observed that industrial effluents and groundwater include the well-known cytotoxic and carcinogenic elements, viz. trivalent arsenic [As (III)], and hexavalent chromium [Cr (VI)], which contribute to the production of reactive oxygen species (ROS), leading to various kidney diseases. Although the compounds mentioned above have been investigated for their toxicities, little is known about their combined effects, particularly concerning the cellular stress response and toxicity mechanism. In light of the above information regarding the environmental assessment of these heavy metals in recent decades, mitigation steps are very much needed to safeguard the biotic system. Biochanin-A (BCA) and phloretin (PHL) are natural compounds found plenty in nature and are known to have free radical scavenging and antioxidant properties. The current study, conducted with unparalleled thoroughness and precision, investigated the potential energizing benefits of these natural compounds against As+Cr intoxicated Swiss albino mice. This work quests the underlying mechanism of BCA and PHL through various biochemical experiments, DNA damage analysis, histological outcomes, and gene expression assays. During the experimental procedure, potassium dichromate (75 ppm) and sodium meta-arsenite (100 ppm) were added to the drinking water for two weeks to induce renal toxicity in the mice. These experimental animals were then supplemented with BCA (50 mg/kg) and PHL (50 mg/kg) intraperitoneally as per the body weight of the experimental mouse. The As+Cr-treated mice group showed an increase in metal burden, protein carbonylation, and renal malondialdehyde, along with a decrease in the activity of several antioxidant indicators (catalase, reduced glutathione, glutathione-S-transferase, superoxide dismutase, and total thiol).

In addition to the above parameters, DNA degradation, microscopy observation, and altered SIRT1/NRF2/HO-1/NQO1 gene expressions were performed in parallel and are corroborated with the above findings. Our study reveals that treatment with the selected antioxidants not only attenuated the renal ROS, DNA damage, and histological modifications but also showed potential in upregulating the SIRT1/NRF2/HO-1/NQO1 signaling pathway. These findings underscore the potential of BCA and PHL to be used as therapeutics in kidney ailments associated with heavy metals exposure, offering a significant opportunity for further research and development in the field of nephrology.

Unraveling the links between iron nutritional status and type-2 diabetes mellitus

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Because it is a strong pro-oxidant, iron may play a role in type 2 diabetes mellitus (DM2) by increasing ROS and oxidative stress, which directly contribute to tissue damage, increasing the risk of developing DM2. The occurrence of DM2 is related to elevated ferritin and iron levels. Even in iron deficient conditions, levels of ferritin, an acute phase and iron storage protein, increase during inflammatory processes. This presentation aims to show the relationship between general nutritional status, oxidative stress, iron nutrition and inflammation in obese subjects with or without DM2. Obesity is a type of moderate chronic inflammation, it is related to higher iron deposition levels, where the expression of miR21 and miR122 microRNAs could play a role. The pancreas, intestinal cells, adipose tissue and liver synthesize hepcidin (Hpc), a hormone that regulates iron metabolism. Iron deposits, infections and inflammation induce its synthesis. Patients with DM2 have elevated levels of Hpc (mRNA and proteins), IL6 and ferritin. On the other hand, the enzyme heme oxygenase (HO1) is responsible for the degradation of heme to CO, biliverdin and Fe2+. At its 5' end, this enzyme presents a polymorphism of GT repeats that may be a risk factor for the development of DM2. There is a subpopulation of diabetic subjects with high systemic iron levels who present this polymorphism. Obese subjects with DM2 have high us-CRP, as well as high relative levels of Hpc abundance, TNFα, IL6, NFκB, TLR2 and TLR4. These factors worsen and maintain inflammation and insulin resistance.

From "crowdoxidation" to unexpected annulation reactions and beyond

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Purpose: Our synthetic chemistry laboratory has been involved with studying a variety of synthetic / natural / bioinspired compounds often with chemical biology research goals in mind. We are having continued “fun” with organochalcogenide-containing fluorescent chemical probes and other trace element / “main group” systems. Our laboratory interests complement some TEMA conference outlooks.

Methods: We train and work within the confines of synthetic bioinorganic (metallobiochemistry), main group, bioorganic and analytical chemistry. We will discuss the functional role of Se, S and other elements through the lens of chemosensing. Sometimes the compounds we make are those that detect trace elements and sometimes the probe contains trace elements themselves which, we believe, are not lost from the molecule before they are excreted. Our compounds are almost always fluorescent. Alterations in fluorescence and photophysical characteristics as a function of time / analyte / conditions are important to us; we can try to observe and interrogate biology in new ways through molecules that can be designed to change upon interaction with external stimuli.

Results: Our compounds can detect analytes such as ROS and biothiols. Our biomimetic ligands and compounds are successful at detection; we often find they possess low cell cytotoxicity / biocompatibility. Through scientific collaborations, we determine the potential our “molecular tools” have in (living) cells and organisms such as mice and zebrafish.

Conclusions: We have found analytical success with our synthesized compounds. The findings often help us design next generation versions for further investigations. We consider bridging fundamental aspects of the trace elements to neurogenerative disease and immune system research. We hope to further explore cancer and metabolism research as well.

Selenium metabolism for selenoprotein synthesis- its relation to biological defense and diseases

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The essential trace element “Selenium” plays a significant role in the antioxidative system in vivo. The biological function of selenium is mediated by selenoproteins, which contain selenocysteine (Sec), a cysteine analog that possesses selenium instead of sulfur. Twenty-five types of human selenoproteins have been identified, including glutathione peroxidase (GPx) for the reduction of hydroperoxides and thioredoxin reductase for redox regulation. Selenoprotein P (SeP) is a major selenium-containing protein in human plasma that is primarily synthesized in the liver. SeP functions as a selenium transporter to deliver selenium from the liver to other tissues, such as those of the brain and testis, playing a critical role in selenium metabolism and antioxidative defense.

SeP transports selenium to the cells via multiple steps, that are first incorporated by SeP receptors such as ApoER2, carried to lysosome, and degraded to Sec. Generated Sec is further cleaved by Sec lyase to form inorganic selenium, which is the substrate of selenophosphate synthetase 2 (SEPHS2) to produce selenophosphate from inorganic selenium and ATP. Further, selenophosphate is used for the synthesis of Sec on tRNA. Recently, peroxiredoxin 6 (PRDX6) has been identified as the acceptor of inorganic selenium, which effectively promotes selenoprotein biosynthesis.

In this presentation, I focus on the physiological role of selenium metabolism, particularly PRDX6 and SeP, in antioxidative defense and protection against environmental stresses. On the other hand, recent studies indicate that excess SeP exacerbates glucose metabolism and cancer. Thus, this presentation also represents the bifacial properties of selenium metabolism regulated by SeP, namely significant role in maintaining homeostasis and its dysregulation as the target of cancer treatment.

Effects of salinity on the transcriptional regulation of trace metal transporters in tilapia

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Variations in salinity are among the main physical parameters that drive the capacity of fish to survive in a range of environments. Many euryhaline teleost fish inhabit waters in which salinity varies tidally between that of fresh water (FW) and seawater (SW), such as estuaries. Acclimation to such environmental changes is dependent on the concerted extrusion and uptake of ions via osmoregulatory tissues, including gill and kidney. With an emphasis on manganese, zinc and iron, this study focused on the transcriptomic responses of specific trace metal transporters of the solute carrier superfamily (SLC) in the euryhaline Mozambique tilapia, *Oreochromis mossambicus*, acclimated to either FW or SW. Branchial transcripts from fish acclimated to steady-state salinities were also compared with those from fish transferred to a simulated tidal regime (TR), which alternated between FW and SW every 6 h. Concentrations of manganese were higher in gills of SW-acclimated fish. In muscle, manganese and iron contents were higher in FW-acclimated fish, whereas zinc was higher in SW-acclimated fish. While only one trace metal transporter, slc41a1, was found to be differentially expressed between FW and SW fish in gill, there were 8 branchial slc transcripts of the trace metal family differentially expressed between fish reared in SW and TR, including members of slc30a, slc39a and slc49a. These data show salinity-dependent changes in tissue manganese, zinc and iron and transcriptional activation of trace metal transporters. Notably, additional transcripts were differentially expressed when fish were transferred to a TR, suggesting that the regulation of divalent cation transport contributes to the capacity of fish to compensate for frequent changes in external salinity.

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Selenosugars, and selenium metabolomics and metabolism from deficiency to high selenium status

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We used high pressure liquid chromatography (HPLC) coupled with Se-specific inductively coupled plasma mass spectrometry (ICP–MS) and molecule specific (ESI Orbitrap MS/MS) detection to study the increase in liver Se in turkeys and rats supplemented as selenite with high-Se (5 μg Se/g diet) and adequate-Se diets. We found that far more Se is present as selenosugar (seleno-*N*-acetyl galactosamine) than is present as selenocysteine (Sec) in true selenoproteins. In high Se liver, the increase in liver Se was due to both low molecular weight (LMW) selenometabolites as glutathione-, cysteine- and methyl-conjugates of the selenosugar, but also as high molecular weight (HMW) selenosugars apparently decorating general proteins via mixed-disulfide bonds. There was no selenomethionine (SeMet) detected in liver when rats or turkeys were supplemented with inorganic selenite up to 5 μg Se/g diet. In rats supplemented with SeMet, SeMet is efficiently and rapidly metabolized to mix with the common Se metabolite pool, where Se is preferentially incorporated into Sec and Sec-selenoproteins until selenoproteins plateau; with high SeMet intake, Se is increasing accumulated as LMW selenosugars and as selenosugar-decorated proteins.
To further demonstrate selenosugar binding to proteins, and to develop tools to more thoroughly characterize the nature of these proteins, aqueous liver extracts from animals fed Se-adequate and high-Se were subjected to SDS-PAGE and Native-PAGE with and without pretreatment with β-mercaptoethanol (βME). The separated proteins were then electrophoretically transferred to membranes, and the membranes subsequently were subjected to laser-ablation inductively-coupled mass spectroscopy (LA-ICP-MS) analysis of ^{78}Se profiles. We found that without βME treatment, ^{78}Se was widely distributed across the protein molecular weight profile for both SDS-PAGE and Native-PAGE of turkey and rat liver extracts, whereas βME pretreatment dramatically reduced ^{78}Se binding, reducing the profile to true Sec-selenoproteins. This reduction was ~50% for both high-Se rat and turkey extracts. The increased ^{78}Se in non-βME treated samples was distributed across the full profile. The use of LA-ICP-MS indicates that selenosugar residues are bound to protein subunits of multiple sizes, and that targeted attachment of selenosugars to a single or limited number of protein subunits does not occur.

Associations of trace elements with reproductive disorders

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Purpose: Studies have suggested the association of trace elements with various reproductive disorders. We aim to investigate the relationship between serum levels of trace elements and various reproductive disorders.
Methods: A total of 102 hyperprolactinemia (>100ng/ml serum prolactin levels), 68 primary testicular failures, and 13 spermiation defect patients were included in this research, besides 4 cases of dark semen with primary testicular failure. Serum and/or semen Cadmium (Cd), Chromium (Cr), Manganese (Mn), and Lead (Pb) levels were analyzed using the Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) method. Elemental electron microscopy was used to study seminal cells in spermiation defect besides all other metals in serum & semen.
Results: No significant correlation was found between prolactin and heavy metals serum levels. In spermiation defect cases, platinum in seminal cells was high in 4 cases. High (four times or more) serum level of lead and nickel was observed in 11 (85%) and 6 (46%) cases of spermiation defect, respectively. Electron microscopy in dark semen cases showed the presence of platinum in all the cases, whereas ICP-AES revealed increased levels of lead, manganese, and nickel in serum.
Conclusions: Our results suggest that there may not be an association between hyperprolactinemia and trace elements. However, we found that high serum concentrations of trace elements like lead and nickel, or high platinum accumulation in seminal cells, could be underlying etiologic factors in human spermiation defects. This unique insight contributes to our understanding of reproductive disorders and opens new avenues for further research.

Boron as additive protects sperm motility and mitochondrial membrane potential during cryopreservation of Jersey semen

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The impact of boron (B), a vital element essential for various biological processes has not been explored for improving reproductive health of the mammals. Earlier study from this laboratory revealed positive impact of B on sperm output and motility, in the present study, protective effect of B as additive in the Jersey semen extender has been explored. The Jersey semen samples (n=8) were collected using artificial vagina and diluted in Tris-based extender (80 million sperm /ml). The diluted semen samples were divided into four groups, control and three boron-treated groups (0.3, 0.45, and 0.6 µg/100 µl) and filled in 250µl French mini straws and subsequently cryopreserved in liquid nitrogen as per the established protocol. After one week of cryopreservation, the semen samples were thawed at 37°C for 30 sec and subjected to functional parameter analysis. The sperm kinematics were analysed using computer assisted semen analyzer. The sperm mitochondrial membrane potential (MMP) and reactive oxygen species (ROS) were assessed using JC-1 and DHR 123 dyes, respectively employing flow cytometry. B at 0.45 µg/100 µl in semen extender significantly enhanced sperm total motility ($p<0.01$) and MMP ($p<0.03$) as compared to control group. The ROS level increased significantly at higher doses of B (0.45 and 0.6 µg/100 µl) as compared to the control group. The kinematic parameters, including curvilinear velocity, average path velocity, and straight-line velocity did not differ significantly between control and boron-treated groups. The study suggests that B at a dose of 0.45 µg/100 µl during cryopreservation is effective for improving sperm motility and mitochondrial membrane potential in Jersey bulls. However, the effect of increase in ROS, which may be common due to elevated MMP, and field fertility rate need to be addressed to understand the effect of B as additives in the bull semen.

Impact of selenium on pregnancy and childbirth in association with thyroid stimulating hormone and oxidative stress

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Background: An important trace mineral, selenium is essential for thyroid hormone metabolism and antioxidant defense. Enough selenium must be present throughout pregnancy for both the mother's health and the fetus's growth. This study aims to determine how selenium levels throughout pregnancy affect the development of the fetus and its correlations with Thyroid stimulating hormone and oxidative stress.

Methods: 400 pregnant women were recruited from Dakshina Kannada, Kasargod, and Udupi districts. Maternal blood samples were collected during antenatal visits, while cord blood samples were obtained immediately after delivery. A thyroid function test was conducted to assess levels of Thyroid hormones. Selenium levels in the serum were determined using ICP-MS. Antioxidants, nitric oxide, and 8-hydroxy-2’deoxyguanosine(8-OHdG) levels were measured using a commercially available kit. Birth outcomes, including birthweight, gestational age at delivery, and neonatal Apgar scores, were recorded.

Results: The data revealed that the study population's maternal and cord blood selenium levels were within the expected range. Low Selenium was observed in hypothyroidism, and it also compared the baby's weight, and Apgar scores, to observe the impact of Selenium on the child.

Conclusions: This study showed Selenium level was equally important in comparing with the baby's weight and thyroid function.

Selenocysteine lyase control of brown adipose tissue physiology and morphology

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Purpose: The role of selenium (Se) in mammalian brown adipose tissue (BAT) adaptive thermogenesis has been classically linked to its presence in the active site of selenoprotein type 2 deiodinase (Dio2) as the amino acid selenocysteine (Sec). Dio2 activates thyroid hormone, necessary for the expression of the mitochondrial proton pump uncoupling protein 1 (Ucp1), responsible for producing heat. Beyond Dio2, understanding of additional mechanisms in which Se controls BAT adaptive thermogenesis, especially in the mitochondria, is lacking. We uncovered that disruption of the gene for Sec lyase (Scly), an enzyme that decomposes Sec into selenide for synthesizing selenoproteins, leads to obesity with concomitant localized Se deficiency and increased susceptibility to diet-induced obesity in mice. We hypothesize that, besides control of Dio2 expression, BAT Scly participates in redox homeostasis via control of glutathione peroxidases (GPXs) and maintenance of mitochondrial structure. **Methods:** Mice with whole-body and BAT-specific disruption of Scly (Scly KO and BAT-Scly KO) were fed diets with low (0.08 ppm) and adequate (0.25 ppm) levels of sodium selenite for 8 weeks and exposed to 4ºC for 6 hours before extraction of BAT for analysis of gene and protein expression and activities, and mitochondria morphology using electron microscopy. **Results:** We found that Scly KO mice have reduced Dio2 and ferroptosis-regulator glutathione peroxidase 4 (GPX4) expression, and activity of Dio2 but not of GPXs. Lower levels of GPX4 did not activate GPX4-independent ferroptotic pathways. Moreover, adipocyte mitochondria of adult Scly KO mice were smaller and with fewer cristae, and this morphological impairment was also detected in newborn mice. Se deficiency, in general, aggravated these outcomes. **Conclusion:** Scly sustains the expression of key selenoproteins needed for thermogenesis and mitochondrial ultrastructure, which implicates this enzyme in the control of cold-induced heat production in the mouse BAT.

Beneficial effects of fungal iron supplementation on gut health

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Background: Excess unabsorbed iron in the gut may increase the growth of enteric pathogens and increase the incidence and severity of infectious diseases. Reduced growth of the common Gram-negative pathogen, *Salmonella* Typhimurium (*S.* Typhimurium) with iron-enriched *Aspergillus oryzae* (*Ao*-Fe) compared to $FeSO_4$ was observed under *in vitro* conditions.
Objective: To compare the effects of oral ingestion of *Ao*-Fe and $FeSO_4$ on growth of *S.* Typhimurium in stools collected from children and women of reproductive age (WRA).
Methods: Children aged 5-12 y and WRA collected a baseline stool sample and then consumed a muffin containing blue dye and 54-65 mg elemental iron as either $FeSO_4$ or *Ao*-Fe. After collecting stools until blue dye disappeared, and then following a 1-week washout period, participants crossed over to the other arm. Stool iron content was determined by ICP-MS. Stool samples were inoculated with *S.* Typhimurium and growth was determined over 12 h. Muffins containing no iron, $FeSO_4$, or *Ao*-Fe underwent the INFOGEST *in vitro* digestion protocol to determine the growth of *S.* Typhimurium.
Results: Stool iron concentrations increased from baseline following ingestion of supplemental iron in children and WRA ($P<0.0001$) but did not differ between iron sources ($P>0.05$). *S.* Typhimurium abundance was greater in stool samples following ingestion of $FeSO_4$ compared to baseline in children ($P<0.01$) and WRA ($P<0.05$), but not between two treatments ($P>0.05$). Increased bacterial growth was observed following the oral and gastric digestion phases of the INFOGEST protocol with $FeSO_4$ compared to *Ao*-Fe; however, growth was similar following the intestinal digestion phase.
Conclusions: As expected, iron supplements substantially increase the amount of unabsorbed iron in the gut. Compared to $FeSO_4$, the *Ao* matrix may withhold iron from enteric pathogens in some parts of the gastrointestinal track, but more studies are needed to confirm these results with low iron fortification doses.

Copper in vascular health and disease

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Atherosclerosis is the major pathological development that leads to cerebral and cardiovascular events and associated mortality. Progression of this condition implicates heavily vascular smooth muscle cells (VSMCs) and the processes closely related to oxidative stress, inflammation, and remodeling of extracellular matrix (ECM). Copper (Cu), an essential microelement, participates in these processes, however its involvement in pathophysiology of VSMCs remains poorly investigated.

In the present study we analyzed Cu impact on VSMC pathophysiological responses in the model of primary VSMCs from human aorta. Using multiple imaging techniques combined with classical biochemical and molecular biology methods we assessed VSMCs functional responses: apoptosis, metabolism, migration and calcification.

Novel data addressing impact of Cu modifications on metal distribution and morphological changes of VSMCs are presented. These morphological changes are associated with the role of Cu in maintenance of cellular metabolism, cell migration, and VSMC calcification. RNA-seq results indicated that high calcium and phosphate treatments activated the pathways related to oxidative stress and inflammation in VSMCs at the initial stage of calcification. Supplementation of calcifying cells with Cu prevented most of the transcriptomic alterations induced by high calcium and phosphate while chelation-mediated restriction of Cu greatly aggravated them.

In summary, acquired knowledge of early phenotypic alterations triggering vascular pathological transformation will allow reassessment of Cu supplementation in management atherosclerosis and other cardiovascular diseases.

Unlocking Hidden Potential: Exploring novel selenoenzyme and unique selenium metabolic pathway in bacteria

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Selenium is utilized by bacteria and archaea in the forms of selenocysteine (Sec) in selenoproteins and selenouridine in tRNA. In addition, some bacteria can use selenium oxyanions (selenate and selenite) as electron acceptors in anaerobic respiration. Most selenoproteins characterized in bacteria so far, such as glycine reductase and formate dehydrogenase, are involved in anaerobic energy metabolisms. Considering the vast diversity of microbial genomes, there must still be a significant number of uncharacterized selenoenzymes/selenoproteins, as well as undiscovered selenium metabolic pathways. In this presentation, I introduce a newly characterized selenoenzyme, multiheme cytochrome *c* selenoprotein (MccSep), and a novel selenium metabolic pathway for C1 assimilation utilizing trimethylselenonium ion (TMSe).

The reduction of elemental sulfur/polysulfide to sulfide is an essential process in the natural biogeochemical sulfur cycle. However, little is known about the enzymes catalyzing sulfur reduction in sulfur-reducing bacteria. We revealed that MccSep is a polysulfide reductase involved in dissimilatory sulfur reduction in *Geobacter sulfurreducens* PCA. MccSep is a tetramer, with each protomer containing five *c*-type hemes and a Sec residue essential for its catalysis.

In animals, nutritional levels of selenium are excreted in the urine mainly as a selenosugar, while excess amounts of selenium are excreted as TMSe. TMSe released into the environment is believed to be degraded into volatile dimethylselenide by soil microbes, impacting the biogeochemical selenium cycle. However, the TMSe degradation process remains largely unknown. Recently, we succeeded in isolating soil microbes that grew on TMSe as their sole carbon source. These microbes were classified into the genus *Aminobacter*, a member of the methylotrophs. TMSe. Through genomic, genetic, and biochemical studies, we identified and characterized a gene cassette for TMSe degradation in *Aminobacter spp*.

Probing biological copper in the subfemtomolar range

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Copper is an essential trace element that plays a critical role in human health. As a redox-active cofactor in metalloproteins, copper catalyzes a broad range of reactions that play pivotal roles in many fundamental biological functions, including respiration, superoxide detoxification, degradation of amines, or the mobilization of iron. Increasing evidence suggests that mammalian cells maintain a kinetically labile pool of monovalent copper(I), which is tightly buffered in the subfemtomolar concentration regime. To devise high-affinity chelators suitable for probing the subfemtomolar activity of cytosolic Cu(I), we created a new family of ligands based on phosphine-sulfide-stabilized phosphine (PSP) binding motifs. Comprised of molecular architectures with varying degrees of conformational flexibility, the chelators offer tunable Cu(I) dissociation constants from the femto- to subzeptomolar range while effectively discriminating against other biologically relevant trace metals such as Zn(II), Fe(II), and Mn(II), even at millimolar concentrations. Taking advantage of this suite of PSP ligands, we determine the Cu(I) affinity of a metallochaperone and probed the thermodynamic availability of Cu(I) in mammalian cell lysate by size exclusion chromatography integrated with ICP-MS for precise elemental quantification. Moreover, integrating a PSP-binding motif within a donor-acceptor fluorophore platform yielded a Cu(I)-selective emission-ratiometric probe, crisp-17, for visualizing dynamic changes in intracellular copper availability by two-photon excitation microscopy. Altogether, these studies paint a unified picture where cells maintain a dynamic yet tightly controlled copper pool that is buffered at low attomolar levels through a polydisperse buffer of bioligands.

Analytical tools for trace elemental analysis at high accuracy and precision

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High-end analytical mass spectrometry techniques, such as inductively coupled plasma mass spectrometry (ICP-MS), MC-ICP-MS, & IRMS, have emerged as indispensable tools for the accurate and precise determination of trace elements in biological samples. These techniques offer unparalleled sensitivity, selectivity, and dynamic range, enabling the detection and quantification of elements at ultratrace levels.

This presentation will discuss the vital roles of trace elements in human and animal health, highlighting their involvement in various biological processes, early disease identification, and bio-forensics. Furthermore, it will focus on applications related to high-end analytical mass spectrometry techniques in trace element research, emphasizing their contribution to advancing our understanding of trace element and related health implications.

Non-canonical roles and mechanisms of trace metal-containing redox proteins

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Se-dependent glutathione peroxidase-1 (GPX1) and copper-zinc superoxide dismutase 1 (SOD1) are major intracellular redox enzymes. However, several metabolic phenotypes resulting from knockouts of these genes in mice cannot be simply explained by direct mechanisms of redox biology. Therefore, we have applied whole animal models, cell culture models, and various cell biology and molecular tools including protein complementation assay (PCA) to reveal non-redox roles of these proteins such as protein-protein interactions (PPIs). We have demonstrated that overexpressing GPX1 down-regulates transcription of regenerating islet protein-2 (REG2) that binds a glycoprotein of voltage-dependent Ca2+ channel (CaV1.2) on the cell surface of pancreatic beta cells. This new cascade affects the glucose-stimulated insulin secretion. Subsequently, we have found the PPIs between SOD1 and tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta (YWHAZ) or epsilon (YWHAE). We have used site-directed mutagenesis of SOD1 to characterize the binding interaction sites of the two PPIs. We also determined the impact of PPIs on lipid metabolism and cell growth and survival of HEK293T and HepG2 cells. The formation of SOD1 and YWHAE or YWHAZ protein complexes elevated enzyme activity of purified SOD1 in-vitro by 40% ($P < 0.05$) and protein stability of over-expressed intracellular YWHAE (18%, $P < 0.01$) and YWHAZ (14%, $P < 0.05$). These PPIs were associated with lipolysis, cell growth, and cell survival in HEK293T or HepG2 cells. In conclusion, revealing the unorthodox roles and mechanisms of GPX1 and SOD1 will provide new perspectives and insights for understanding their functions and for diagnosing and treating diseases related to these and other antioxidant proteins.

Biomimetic Studies on Metal Homeostasis and Detoxification

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Methylmercury ($Me–Hg^{+}$) is an extremely neurotoxic and ubiquitous environmental pollutant, which accumulates at high levels in food chains, mainly in fish and seafood, and therefore, consumption of contaminated foods poses a significant risk to human health. Exposure to ethylmercury ($Et–Hg^{+}$) is another serious concern in developing countries where $Et–Hg^{+}$-containing antimicrobial agent "*Thimerosal*" is commonly used as a preservative in multiuse vials of vaccines and other medicines. In nature, however, several microorganisms have been reported to detoxify organomercurials, including $Me–Hg^{+,}$ by converting them to less toxic biologically inert species. For instance, bacterial organomercurial lyase (MerB) catalyzes the protolytic cleavage of the otherwise inert Hg–C bond of $Me–Hg^{+}$ and produces methane (CH_4) gas and less toxic Hg^{2+}, while a second enzyme mercuric ion reductase (MerA) subsequently reduces Hg^{2+} to volatile Hg^{0}.

Likewise, arsenic is another ubiquitous environmental toxin and human carcinogen that also poses a serious threat to human health.[3,4] However, in this case, methylation of inorganic arsenic (iAs) is recognized as an established detoxification process in organisms. S-adenosylmethionine (SAM) is a universal cofactor that methylates iAs(III) up to three times, with the help of As^{3+} S-adenosylmethionine methyltransferase, producing the less toxic methylated arsenic species, including dimethylarsenite ($DMAs^{3+}$).

On the other hand, copper ion is essential in our lives and used as a cofactor in several vital processes. However, high concentrations of copper can be deleterious as they promote Fenton-like reactions, thereby increasing the cellular ROS levels and leading to the oxidative damage of cellular constituents like proteins, lipids, and nucleic acids. Thus, it is essential to control copper concentration in the body strictly. This talk will focus on the homeostasis of copper and the detoxification pathways of mercury and arsenic-related compounds in the cellular system.

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Selenium and chromium in animal nutrition: Unveiling complex role in stress resilience, health and production

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Despite its previously known toxic properties, selenium is a beneficial essential trace element that helps alleviate stress. Our research using rat and goat models has shown that higher levels of dietary selenium effectively reduce both biotic and abiotic stress. At elevated dietary levels, selenium can mitigate stress induced by LPS and heat caused by high ambient temperatures. The multifaceted beneficial effects of dietary selenium include modulation of blood and biochemical parameters, protection of the liver, modulation of cytokine response and immunity, alteration of mineral metabolism in the host, and expression of essential selenoprotein genes involved in antioxidant action and heat shock protein. In goats, higher dietary selenium also improved meat quality and reproductive attributes under endotoxin-mediated stress conditions and improved thermoregulatory parameters such as respiration and body temperature of goats under heat stress. Similarly, chromium has demonstrated positive effects in managing environmental, dietary, and physiological stresses across different dietary levels. Its supplementation in organic forms like chromium picolinate and chromium propionate has been linked to better antioxidant status, improved ruminal metabolism, and higher protein utilization efficiency in heat-stressed dairy cows. Improved antioxidant levels and the health response of LPS-challenged offspring born to ewes supplemented with chromium showed its potential for nutritional programming. Additionally, chromium picolinate has shown potential in reducing broilers' gut pathogenic microbial load under heat stress and normal conditions, and chromium propionate has demonstrated improved feed efficiency and meat attributes in boilers. Chromium is also noted for altering rumen microflora to favour fibre-degrading and acetogenic bacteria and supporting macrophage development and activation. This review sheds light on the latest advancements in understanding selenium and chromium's complex roles in promoting biotic and abiotic stress resilience, thereby improving animal health and production outcomes.

Pathophysiological analysis of metaphyseal dysplasia in a selenoprotein GPx4 deficient mouse model

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GPx4 (phospholipid hydroperoxide glutathione peroxidase) is one of the selenoproteins, that directly can reduce phospholipid hydroperoxide produced in lipid memberane by oxidative stress. GPx4 is reported as a regulator for ferroptosis, an iron dependent lipid peroxidative cell death. We first previously found that depletion of GPx4 induce embryonic lethal at 7.5dpc and depletion of GPx4 induce slowly cell death in MEF cells (1). Recently, we reported that slowly progressive cell death, Lipoxytosis, induced by GPx4-deficiency occurs via MEK1/ERK2 activation as a downstream signal after iron independent lipid peroxidation (2-4).

Sedaghatian-type and Patterson-Lowry type -metaphyseal dysplasia are lethal rare diseases that cause skeletal deformity and central nerve malformation. In this study, we screened Sedaghatian-type and Patterson-Lowry type -metaphyseal dysplasia patients' whole genomes and found three single amino acid mutants and two splicing abnormalities of GPx4 in several patients. Then we transfected these human mutant GPx4 cDNA and splicing abnormal variants to tamoxifen inducible GPx4 depleted MEF cells and evaluated that these mutant GPx4 could lose the GPx4 activity and could not rescue GPx4 depleted cell death. To elucidate the role of GPx4 in bone formation we generated cartilage-specific GPx4 KO mice. Cartilage-specific GPx4KO mice failed to grow immediately after birth and lethal in approximately 4 days. A marked decrease in cartilage maturation cells were observed in cartilage-specific KO mice.

In conclusion, loss of function by these mutant GPx4 in cartilage induces metaphyseal dysplasia. Now we are currently using these mice to develop therapies.

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Selenization of the small molecule drug structures

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Purpose: Our studies and literature evidence have shown that the position of the selenium (Se) atom, in addition to other structural features of a small molecule, dictates the overall potency of a compound. This is particularly true regarding their application as cancer therapeutics as many, albeit not all, newly designed Se molecules have shown promising therapeutic efficacy in preclinical cancer models. Furthermore, a large body of our studies has shown that the isosteric replacement of a sulfur (S) atom in a small molecule by selenium can lead to more cytotoxic compounds having the potential to be developed as cancer therapeutics. Over the years, we designed several Se-drug compounds by rationally incorporating Se into various drug structures including temozolomide, NSAIDs, biotin, and kevetrin among others, and assessed their efficacy and toxicity using different preclinical cancer models.

Methods: The selenization of drug structures was conducted using customized synthetic techniques and their toxicity and efficacy were determined using well-established cell culture and in vivo cancer mouse models.

Results: In most cases, the lead molecules from structure-activity relationship (SAR) studies of each series of Se-drug compounds developed were 10-500-fold more potent than the parent drug in inhibiting cancer cells' growth, inducing apoptosis, and inhibiting xenograft tumor growth. Systemic toxicity was also observed to be increased in most cases but could be manipulated by lowering the dose due to substantially lower dose requirements owing to the enhanced potency and/or incorporating the Se-drug into a nanoformulation.

Conclusions: Our studies demonstrate that the appropriate incorporation of Se into small molecule drug structures can significantly enhance anticancer activity, and thus strategically incorporating Se could be a valid avenue to create future cancer drugs.

Importance of trace elements and their characterization techniques in different high temperature alloys

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Gas Turbine hot section components are predominantly made of Nickel based and Cobalt based superalloys. They are an engineering marvel by having the alloy composition with more than 13-14 elements from the periodic table, each playing an indispensable role in their functionality in terms of creep, fatigue, hot corrosion and fracture, all at high temperatures. This talk will focus on the role of trace elements such as Boron, Carbon, Niobium, Rhenium etc. in Nickel based alloys and the advanced characterization techniques to establish their role in the evolution of newer alloys for advanced manufacturing of turbine components.

Heavy metals induced toxicity among E-waste workers: A cross-sectional study

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The electronic waste (e-waste) is currently the largest growing waste stream as the use of electronic equipment has increased dramatically over the last few decades and is a global environmental health hazard. In developing countries, the unskilled collection techniques as well as inappropriate facilities causes human health and environmental complications. Through these unofficial recycling, valuable metals such as copper, iron, gold, silver, aluminium and platinum are recovered. The e-waste is composed of a variety of hazardous, toxic and allergenic metals as well as chemicals. Common toxic metals include Antimony, Arsenic, Germanium, Cadmium, Chromium, Cobalt, Indium, Lead, Mercury, Nickel, Thallium and variety of rare elements. To assess heavy metal induced toxicity among e-waste workers, a cross-sectional study was conducted in Bangalore, Karnataka. A total of 215 workers (mean age 27.16±3.22 years) employed in an e-waste processing unit participated in this study consisting of e-waste recycling workers (n=144) and supporting staff (n=71). We estimated heavy metal concentration, assessed hematological, biochemical and genotoxicity parameters and determined urinary 8-Hydroxy-2'-Deoxyguanosine (8-OHdG) levels. A questionnaire containing demographic information, medical history, lifestyle characteristics, health complaints, and occupation-related information was used after obtaining informed consent. The hematological parameters were normal, except for microcytic hypochromic and normochromic anemia in ~8% and 11.5% workers, respectively. The comet assay showed high frequency of DNA damage index among study participants. Significant increase in 8-OHdG levels and abnormal levels of SGOT and SGPT were observed. Additionally, chronic health complaints such as asthma (1.4%), hypertension (6.9%), and diabetes mellitus (3.5%) were also observed along with other health complaints. The blood and urine concentrations of arsenic, cadmium, chromium, manganese, nickel, and lead in e-waste workers were significantly higher ($p<0.05$) as compared to e-waste supporting staff. Overall, this study emphasizes the need for regularly monitor the burden of heavy metals among e-waste workers and design effective measures to prevent harmful work environment caused by personal exposure to toxic heavy metals.

Posters

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Assessing nutrient retention and bioavailability in biofortified millets: A focus on low-temperature processing

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Millets are a staple crop for millions of people in arid and semi-arid regions due to their resilience to climatic conditions and high nutritional content. Recent trends in biofortification have focused on enhancing the micronutrient profile of millet to help alleviate malnutrition. However, nutrient degradation during processing is a significant concern, as many traditional processing methods pose nutrient losses; likewise, mineral bioavailability in millet variety is a major area of concern due to the presence of antinutrients such as phytic acids, polyphenols, and fibers. Low-temperature processing offers an alternative that potentially preserves more of these essential nutrients. This study aims to evaluate the effectiveness of low-temperature techniques in retaining nutrients and improving bioavailability in biofortified millets. A selection of biofortified millet varieties, enriched in iron, zinc, and B vitamins, will be processed using three low temperature methods: low thermal plasma, cold extrusion, and vacuum drying. These will be compared to traditional milling, roasting, and puffing methods. Nutrient retention will be analyzed using spectrophotometry for iron and zinc, calcium, magnesium, and high-performance liquid chromatography (HPLC) for vitamin content. An in vitro digestion model will be employed to simulate human gastrointestinal conditions and assess post-process nutrient bioavailability. Statistical analysis will be performed to determine significant differences in nutrient retention and bioavailability between processing methods. The study suggests that low temperature processing can enhance both the retention and bioavailability of essential nutrients in biofortified millets. The findings of the investigation have significant implications for improving the nutritional quality of millet-based diets in regions where micronutrient deficiencies are prevalent. Adopting low-temperature processing at the industrial and household levels could play a key role in improving the health outcomes of populations dependent on these crops.

Role of staple food fortification in combating micronutrient deficiency

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Micronutrient deficiencies, often referred to as "hidden hunger," result from the lack of essential nutrients and minerals in the diet that are necessary for cellular and molecular functioning. According to the World Health Organization, micronutrients are required in amounts less than 100 mg per day. In India, over 70% of the population consumes less than half of the recommended daily allowance of these essential nutrients, resulting in significant economic impact. Micronutrient deficiencies contribute to malnutrition, leading to the deaths of approximately 6,000 children under the age of five in India every day, with more than half of these deaths linked to deficiencies in essential micronutrients like iron, zinc, iodine, and folic acid. During pregnancy and lactation, a higher serum level of calcium and other minerals are required for nourishment and if calcium in the diet is insufficient to meet this extra demand, the health of the mother and baby may be compromised because of bone loss from the maternal skeleton, reduced fetal growth and bone mineralization and impaired breast-milk calcium secretion.

Fortification of staple foods has emerged as a proven, cost-effective and sustainable way of providing vital nutrients to a large population and involves the intentional increase in the content of specific vitamins and minerals in food products to improve their nutritional quality. This not only prevents deficiencies but also restores nutrients that may be lost during food processing, contributing to improved public health outcomes with minimal risks.

The present work reviews the importance of fortifying edible flours with nano sized micronutrients to achieve improved absorption in the body. Daily consumption of these food products will ensure a steady supply of micronutrients, thus improving overall health.

Quantitative proteomic analysis revealed CD24 dependent modulation of metabolic reprogramming during epithelial to mesenchymal transition in Oral Squamous Cell Carcinoma (OSCC)

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Purpose: Oral cancer is the second most common cancer in India, with rising incidence, especially in the younger ages, making it a socioeconomical burden. Few patients develop aggressive cancer depending on their genetic and molecular makeup. Epithelial-to-mesenchymal transition (EMT), a key event in cancer aggressiveness, involves metabolic rewiring that allows cells to fulfil their metabolic demands. The glycoprotein CD24 is linked to cancer aggressiveness, however, its role in regulating metabolic reprogramming during EMT is poorly understood. In this regard, the aim of our study is to elucidate the role of CD24 status and the trace micronutrient selenium axis in modulating metabolic reprogramming during EMT in OSCC.

Methods: An in-vitro TGF-β induced EMT model was optimized. Cells were harvested on day 6 and the proteome and metabolome were quantified using LC-MS/MS. Label-free quantification was employed to calculate the differential proteome changes. Other relevant experiments were performed to show EMT-specific changes in the cancer cells.

Results: A total of 4135 proteins were quantified across all samples in the oral cancer cell lines. CD24 status and the trace micronutrient selenium axis play a key role during EMT in OSCC cells.

Conclusions: The study examined the influence of CD24 status on the modulation of metabolic reprogramming during EMT in OSCC. Further, targeting selenoproteome and selenoprotein biosynthesis can prove to be potentially useful for cancer therapeutics.

Trace elements in neurodegeneration, unraveling common pathways in Alzheimer's, Parkinson's, and beyond

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The multifactorial and multievent nature of neurodegeneration renders the subject of major challenge. Neurodegenerative disorders including Alzheimer’s disease, Parkinson’s disease, prion disease, Huntington disease share a number of different pathological conditions, which share similar critical metabolic processes, such as protein aggregation and oxidative stress, which are associated with the involvement of metal ions. These conditions highlight common trends that could inform therapeutic strategies. Chelation therapy can be considered as a promising approach that target metal ions that may contribute to disease pathology. Chelation therapy could potentially mitigate oxidative stress and protein aggregation by removing excess metals or restoring homeostasis and thus offering a rational approach for designing new therapeutic agents aimed at treating neurodegeneration. Continuous research into the relationship between metal ions and neurodegenerative diseases is crucial for developing effective treatments.

Development of a zinc-based barrier vaginal contraceptive: Ex-vivo and in-vivo study

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The aim of the present study was to formulate and evaluate the reversible vaginal contraceptive suppositories containing zinc acetate as a spermicidal agent. These suppositories were developed by fusion method using glycerol-gelatin and polyethylene glycol (bases), croscarmellose and sodium starch glycolate (super disintegrating agent). All formulations were evaluated for appearance, disintegration time, uniformity mix, flexibility and rigidity of preparation, formation of plug etc., Based on these parameters, two glycerol-gelatin formulations were selected for further studies. Selected formulations were subjected for spermicidal content estimation, microbial load determination was carried out. *Ex-vivo* spermicidal activity and *in-vivo* contraception potential were determined. Formulations were also subjected to stability studies as per standard protocols. Based on the obtained results, CS6 and CS10 Formulations with disintegration time of 4min and 6min respectively selected for further studies. Percentage spermicidal content was found to be within 98 – 102%. No microbial growth was observed during microbial load determination study. These formulations permanently immobilized spermatozoa cells within 20 seconds of coming in contact and revealed different death patterns of spermatozoa cells. Revival of spermatozoa cells did not occur killing them permanently. *In-vivo* rate of contraception was observed to be 100% and 84% for CS6 and CS10 formulations respectively. Stability studies did not show any significant changes in the formulations. Based on these observations, we concluded that suppository formulation CS6 was more effective than CS10. These studies also warrant further in-depth characterization.

Dissecting the role of selenoprotein W in the pathogenesis of tularemia

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Selenium (Se) is an essential trace element incorporated into proteins as the amino acid selenocysteine. Selenoproteins, which contain one or more selenocysteine residues, play crucial roles in optimal immune functions. Both Se and selenoproteins offer protection against bacterial infections. The mechanistic basis for these beneficial effects of Se and selenoproteins is not well understood. To investigate the specific effects of host selenoproteins during bacterial infection, we examined the interaction between the host and *Francisella tularensis* (*Ft*) a gram-negative intracellular bacterium that causes tularemia in humans and animals. The *Ft* genome lacks the machinery for selenocysteine synthesis making it an ideal model for studying host Se and selenoprotein regulation.

RNA sequencing of *Ft*-infected macrophages revealed differential regulation of a protein of unknown function, Selenoprotein W (SelenoW). SelenoW-deficient mice recovered faster after intranasal infection with *Ft*. Consistent with these *in vivo* results, SelenoW-deficient bone marrow-derived macrophages harbored fewer bacteria than the wild-type. These findings suggest that SelenoW may promote bacterial growth in macrophages through an as-yet-unknown mechanism.

Unlike other bacteria, where autophagy is detrimental to growth, *Ft* benefits from autophagy by deriving nutrients to be a successful pathogen. It is known that selenium and selenoproteins can influence autophagy. Therefore, we hypothesized that selenoproteins might disrupt autophagy and, consequently, affect *Ft* burden. Our results suggest that knocking out SelenoW prevents the endosomal escape of *Ft* into the cytosol and reduces autophagy, which in turn makes the bacteria more susceptible to killing within macrophages. This finding implies that SelenoW may potentially exacerbate the disease. Our current work is focused on understanding the mechanism by which SelenoW affects endosomal trafficking and autophagy during infection. Overall, these studies will help us understand how different selenoproteins influence host-pathogen dynamics and may reveal novel targets for controlling infection and pathology.

Effect of Organic Trace Minerals at Different Dietary Levels on Nutrient Digestibility and Trace Mineral Balance in Pigs

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A digestion trial was conducted to assess the effect of organic trace minerals (TM) (proteinates of Cu, Zn, Mn, Fe and Se) at different dietary inclusion levels on nutrient digestibility and trace mineral balance in pigs. The dietary treatments consisted of a basal diet supplemented with inorganic TM at 100% recommended levels (ITM 100) or with organic TM at 100 % (OTM 100), 75 % (OTM 75) and 50 % (OTM 50) recommended levels or with both inorganic TM at 50 % and organic TM 50 % recommended level (ITM 50 + OTM 50). The organic TM employed as proteinate in nature while inorganic TM used were sulphate salts of Cu, Zn, Mn and Fe and sodium selenite. 15 large white Yorkshire pigs aged 5 months were distributed into five groups of three pigs each. Each animal was housed in an independent pen and assigned with one of the five diets in completely randomized design. The duration of the trial included adjustment period for 5 days followed by collection period for 3 days. The dry matter intake and excreta voided was measured during collection period and the samples collected were analysed for proximate principles (AOAC, 2015) and trace minerals (Atomic absorption spectrophotometer). The results revealed that dry matter, organic matter, crude protein, ether extract, crude fiber and nitrogen free extract digestibility were similar among different diets. The daily retention of Cu, Zn and Mn in terms g, g/kg metabolic body weight and % of mineral intake were lower ($P<0.01$ for Cu and Zn; $P<0.05$ for Mn) in OTM 75 and OTM 50 groups. However, the retention of Fe was similar among different groups. It was concluded that inorganic trace minerals in pig diets can be replaced with organic trace minerals at lower inclusion level i.e., up to 50 % of recommended level with better retention of Cu, Zn and manganese and without affecting the nutrient digestibility.

Studies on biofortification and bioaccessibility of zinc and iron in wheatgrass

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To explore the biofortification and bioaccessibility of minerals like Zinc and Iron in wheatgrass. Wheatgrass is 6-10 days old seedling of common wheat (*Triticum aestivum* L.) known for its rich nutrient profile, including minerals, vitamins, polyphenols and enzymes. It is widely consumed as a nutraceutical, primarily in the form of juice and powder. The present study explored biofortification of wheatgrass with zinc and iron using varied concentrations of $ZnSO_4 \cdot 7H_2O$ and FeEDDHA under hydroponic conditions. Wheatgrass was analysed for its mineral accumulation using microwave plasma atomic emission spectrometry (MPAES). Bioaccessibility studies through *in vitro* digestion and dialysis method. Mineral uptake was further studied using everted gut sac model in male Wistar rats. The biofortification results indicated nearly four-fold increase in zinc content and two-fold increase in iron content compared to the control. Bioaccessibility studies through *in vitro* digestion and dialysis method indicated increased quantities of bioaccessible zinc and iron compared to control. The results of mineral uptake through everted gut sac model demonstrated a 14.7% increase in zinc uptake for the zinc-fortified wheatgrass sample compared to the control. Further, bioavailability and efficacy studies shall be conducted in Wistar rat models. These initial findings suggest that wheatgrass can serve as a sustainable and affordable dietary supplement for populations deficient in zinc and iron.

Mitigation of trace metal through plant derive saponin biosurfactant from *Sapindus mukorossi*

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Abstract:

Trace elements exhibit an ambivalent nature in different environments and at varying scales. While they naturally occur in minute quantities, even slight increases in their concentration can lead to detrimental effects on the environment and ecosystems. Despite their vibrant presence, the imbalance caused by their accumulation poses significant ecological hazards. Anthropogenic activities have significantly influenced the formation and distribution of trace elements. Commonly known trace elements, such as copper, iron, zinc, selenium, cadmium, and cobalt, are found in various environments where they can be utilized by microbes and plants through different mechanisms. Some of these trace elements are classified as heavy metals due to their persistence in the environment, requiring complex and specialized processes for their removal. Conventional methods, including physical and chemical approaches, have been widely implemented on an industrial scale. However, the recent development of bioremediation techniques using bio-based compounds has introduced a promising and sustainable approach to mitigating toxic trace metals from the environment. Biosurfactants are notable biocompounds known for their ability to reduce surface tension and enhance emulsification, making them an essential option for the remediation of trace metals from polluted lands. Given that plants are well recognized for producing various bioactive compounds, this study focuses on the extraction of saponins from *Sapindus mukorossi* as a potential biosurfactant for environmental applications.

Selenium ameliorates acetaminophen-induced oxidative stress via MAPK and Nrf2 pathways in mice

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Overdose of acetaminophen (paracetamol), a widely used non-prescriptive analgesic and antipyretic medication, is one of the main causes of drug-induced acute liver failure around the world. Oxidative stress contributes to this hepatotoxicity. Antioxidants are known to protect the liver from oxidative stress. Selenium, a potent antioxidant, is a commonly used micronutrient. Here, we evaluated the protective effect of selenium on acetaminophen-induced hepatotoxicity. Treating Wistar albino mice with sodium selenite (1 mg/kg) before or after inducing hepatotoxicity with acetaminophen (150 mg/kg) significantly reduced the levels of liver injury biomarkers such as serum glutamate oxaloacetate transaminase and serum glutamate pyruvate transaminase. In addition, selenium-treated mice showed decreased levels of oxidative stress markers such as protein carbonyls and myeloperoxidase. Acetaminophen treatment stimulated all three mitogen-activated protein kinases (MAPKs) and Keap1 and decreased the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and heme oxygenase-1(HO-1) in the liver and in isolated mouse peritoneal macrophages, which was reversed by selenium treatment. Our findings suggest that the reactive oxygen species-mediated Nrf2 and MAPK pathways are critical players in acetaminophen-induced hepatotoxicity. These key findings offer an alternative therapeutic target for addressing acetaminophen-induced hepatotoxicity.

Kinetic analysis of TmsA, a key enzyme in trimethylselenonium demethylation that drives environmental selenium cycling

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Selenium (Se) is an essential trace element for animals but becomes toxic at slightly higher levels. Animals detoxify excess Se by adding sugar or methyl groups and excreting the derivatives in urine. One such methylated Se compound, trimethylselenonium ion (TMSe), is degraded by soil microbes to volatile dimethylselenide, which is released into the atmosphere, impacting the biogeochemical Se cycle. We isolated a soil bacterium assimilating TMSe and identified a novel methyltransferase, TmsA, which utilizes TMSe as a substrate and shares 27% amino acid sequence identity with the dimethylsulfoniopropionate (DMSP)-tetrahydrofolate (THF) methyltransferase DmdA. However, how TmsA has adapted to catalyze TMSe demethylation remains elusive. To this end, we performed kinetic analysis to reveal its substrate specificity and catalytic mechanism. First, we examined the substrate specificity of TmsA by measuring the product, 5-CH_3-THF, using HPLC. As a result, TmsA exhibited the highest turnover rate for TMSe, while it showed low activity toward DMSP. Next, structural prediction of TmsA by AlphaFold2 revealed putative catalytic residues (Tyr41 and Glu72). In addition, TmsA was predicted to lack an Arg residue that contributes to DMSP recognition in DmdA, instead forming a narrow substrate binding site by Trp18 and Tyr20. W18A showed a higher K_m value than the wild type, while Y20A showed similar kinetic properties to the wild type. E72A and Y41A showed significantly lower k_{cat} values. Based on these results, we conclude that TmsA is a novel enzyme specialized in TMSe demethylation, with Trp18 involved in substrate recognition, and Tyr41 and Glu72 crucial for catalysis.

The Impact and Role of Iron in biofilm formation of *Candida* species

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Iron plays a critical role in the biofilm formation of various *Candida* species, influencing both the structure and pathogenicity of these biofilms. Biofilms are complex communities of cells embedded in an extracellular matrix, and plays a key factor in persistent infections, particularly in immunocompromised individuals. The role of iron in biofilm formation and maturity is modulated differently in different species of *Candida* such as *Candida albicans*, *Candida krusei, Candida glabrata* and *Candida tropicalis*. These *Candida* species are known to form biofilms on medical devices (such as catheters and prosthetics), contributing to persistent infections. Iron acts as a cofactor for enzymes involved in cellular respiration, DNA synthesis, and oxidative stress response. *Candida* species have evolved sophisticated iron acquisition mechanisms such as siderophore production, reductive iron assimilation, and heme uptake. These mechanisms not only facilitate iron uptake but also contribute to the virulence and biofilm formation of *Candida* species, aiding in its evasion of host immune defences. Few of the studies indicates that iron chelation, or the reduction of bioavailable iron inhibits biofilm development, suggesting that iron is essential for initial cell adhesion and structural stability. Conversely, an abundance of iron promotes biofilm growth and increases the production of virulence factors, such as hydrolytic enzymes, which enhance biofilm resilience. Therefore, this review focuses on understanding the impact and role of iron in biofilm formation of *Candida* species which is essential for developing targeted antifungal strategies that disrupt biofilm stability by modulating iron levels, presenting a promising avenue for managing *Candida*-related infections.

Phytochemical characterization, isolation, and evaluation of wound healing properties in a banana peel extract formulation

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Conventional wound healing treatments often depend on antimicrobials and antibiotics, which can result in limitations, including the rise of antimicrobial resistance. This study aimed to explore natural alternatives by investigating the wound healing potential of banana peel extract. The primary goal was to develop wound dressings incorpora5ng banana peel extract and evaluate their efficacy in promoting skin regeneration. Banana peels were sourced and authenticated from GKVK, Bengaluru, followed by extraction using the Soxhlet method with different solvents. Ethyl acetate was identified as the most effective solvent through phytochemical screening. GC-MS analysis was conducted on the ethyl acetate extract to identify its chemical constituents, and its antibacterial activity was assessed using agar diffusion against gram-positive and gram-negative bacteria. Cytocompatibility was evaluated via the MTT assay on human dermal cell lines, and a scratch assay was used to measure wound healing potential in comparison to a standard marketed product. The wound dressing formulation was optimized by incorporating banana peel extract and assessing parameters such as swelling capacity and pH. The findings suggest that banana peel extract has significant promise as a natural, cost-effective wound healing agent. This research contributes to the development of innovative wound care materials that reduce the reliance on synthetic antimicrobials and promote healthier skin repair.

Method for extraction of heavy metal from dental calculus

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Dental calculus, a mineralized plaque on teeth holds forensic significance as it can estimate an individual's exposure to heavy metals which may serve as toxicological evidence. The varying composition of heavy metal in dental calculus may reveal about the individual's geographic location, occupational exposure, and migratory patterns. Heavy metal exposure at a chronic level becomes health hazardous. Traditionally extracting them from a given matrix mostly uses classical dry ashing method /wet digestion method or its modified forms often involving environmentally harmful chemicals.

The purpose of this research was to find out an alternative ecofriendly method of heavy metal extraction from dental calculus which is less explored. The developed method used extraction of heavy metals from dental calculus by first drying it and crushing the dry mass to fine powder form, followed by addition of digestion solution of hydrogen peroxide, which oxidizes the dental calculus powder and releases the heavy metal in its natural form into the solution. In turn the digestion solution itself gets decomposed into pure water and oxygen which makes this method a promising approach for green extraction chemistry, fulfilling one of the sustainable development goals i.e., SDG of United Nations.

The present research was able to show positive extraction when tested analytically using Inductively Coupled Plasma-Mass Spectrometry method (ICP-MS) demonstrating the viability of this eco-friendly extraction method. This novel method is already published under Indian Patent Office with Patent Application # 202441047199.

It not only advances forensic science but also provides a sustainable approach for analyzing heavy metal exposure through dental calculus.

Development and characterization of zinc rich ready to eat structured supplementary food for children

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To formulate and characterize bioaccessible zinc enhanced ready to eat structured supplementary food for children. Supplementary food mix was formulated, fortified with zinc and bioaccessible zinc was enhanced through processing and additives and sweet and savoury variants were prepared. Structuring of the mix was done using a tabletting machine. The products were evaluated for chemical composition, zinc bioaccessibility, sensory attributes, microbial quality, and flavour profiles. Storage studies were conducted at temperatures of 27 ± 1°C and 37 ± 1°C over a period of 90 days. The structured mix weighed around 5-6g each contained 10 mg of zinc per 100 g, 18-20% protein and 9-12% fat. Bioaccessible zinc of the product was 1.6 mg which is approximately 50% of the RDA for 1-3Y old children. The Heracles II e-nose identified approximately 30 flavour compounds, with (Z)-4-heptenal, 1, 8-cineole, 1-octanol, 3-methylbutanal, trans-2-undecen-1-ol, and p-cymene being the key compounds. Acceptability studies of the product revealed an overall acceptability score of 12 for the sweet variant and 11 for the savoury variant out of 15. The product was found to be microbially safe with the plate count within the Food Safety & Standards Authority of India (FSSAI) limits and devoid of microbial pathogens.

Molecular insights into the potential of eriocitrin to enhance selenium bioavailability

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Eriocitrin, a flavonoid compound, has gained significant attention for its potential health benefits, including its ability to modulate trace element bioavailability. This study aims to explore the molecular mechanisms underlying the interaction between eriocitrin and selenium.

To investigate the potential binding interactions between eriocitrin and selenium, computational techniques were employed. Molecular docking simulations were performed to identify potential binding sites on Selenoprotein P, a key protein involved in selenium transport and antioxidant defense.

The results suggest that eriocitrin could potentially interact with SelP, potentially influencing its function and enhancing selenium uptake. Furthermore, quantum chemical calculations were used to assess the stability of the eriocitrin-selenium complex. By analyzing the electronic structure and energy profile, insights into the strength of the interaction and the potential impact on selenium bioavailability were gained.

These findings provide a deeper understanding of the molecular mechanisms underlying the potential synergistic effects of eriocitrin and selenium and may pave the way for the development of novel strategies to improve selenium bioavailability and overall health.

Thiolated chitosan hydrogel enhanced with chitosan-CuO nanoparticles for wound healing applications

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This study aimed to develop and characterize a chitosan (CS)-CuO nanoparticle (NNP) loaded thiolated chitosan (TCH) hydrogel for potential use in wound healing. Thiolation of CS was achieved through a coupling reaction, and TCH was characterized using FT-IR to confirm thiol group substitution and amide bond formation. CS NNPs were prepared via ion-gelation techniques and characterized accordingly. A molecular docking study was conducted to predict the binding interactions of the hydrogel components with inflammatory mediators involved in wound healing. In addition to physicochemical assessments, such as tensile strength, pH, swelling index, spreadability, and antibacterial activity, we performed MTT and scratch assays to evaluate the hydrogels' antibacterial properties, cell compatibility, and wound healing efficacy. A cell adhesion study was also conducted on the optimized hydrogel to assess its cell adhesion capability. The prepared hydrogels demonstrated significant antimicrobial activity against Streptococcus mutans and Klebsiella pneumoniae. Furthermore, biological evaluations indicated non-toxicity and excellent cell adhesion properties. Overall, this study provides strong evidence supporting the potential of the CS-CuO NNPs loaded TCH hydrogel for effective wound healing applications.

Selenium analogues of α-Lipoic acid – Effect on activation of eNOS and their Antioxidant Properties

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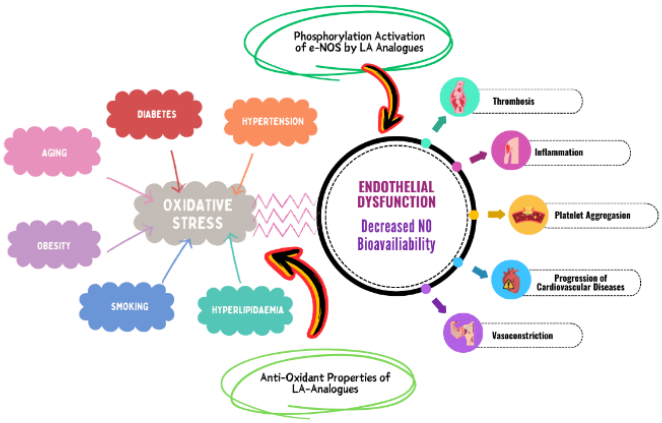
α-Lipoic acid (LA) (thiotic acid or 1,2-dithiolane-3-pentanoic acid) is an organic disulfide found endogenously in plants and animals, used as a dietary supplement to treat diseases associated with oxidative stress. α-LA and its reduced form dihydrolipoic acid (DHLA) exert beneficial effects by acting as biological antioxidant, chelating metal ions, regenerating endogenous antioxidants like vitamin C, vitamin E and glutathione (GSH). Selenium is an important micronutrient and an important biologically functional trace element in our body. It is incorporated as selenocysteine in antioxidant enzymes like glutathione peroxidase (GPx) and thioredoxin reductase (TrxR) and is responsible for maintaining the redox status of the cell. High levels of ROS (Reactive Oxygen Species) due to oxidative stress are known to potentially damage cellular biomolecules and are implicated in the progression and pathogenesis of various chronic diseases like cancer, aging, autoimmune disorders, neurodegenerative and cardiovascular diseases. In this study, we have evaluated the effect of selenium analogues of α-lipoic acid in maintaining the cellular redox status under oxidative stress conditions. Furthermore, LA improves Reduced Nitric Oxide (NO) bioavailability by activating eNOS through activation of PI3-kinase/Akt signaling pathway. Further for the first time, we investigated the effect of selenium analogues of LA on the bioavailability of NO at cellular level.



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Swarna Bindu – An ancient approach to modern disease management

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In the era of regenerative medicine, the application of nanoparticles has paved a new pathway in the treatment of several previously untreated diseases. Metals such as silver, copper, iron, and zinc, in the form of nanoparticles, are widely used in clinical treatment. Among these, gold nanoparticles are gaining considerable popularity due to their positive outcomes. Traditionally, in the Ayurvedic system of medicine, Swarna Bindu is an orally administered method that involves the usage of Swarna Bhasma, that is the gold nanoparticles obtained through a systematic purification process. Swarna Bhasma when incorporated with natural substances such as Ghee – clarified butter, or honey, and administered over a specific period, showcases enhanced respiratory and neural functioning. These gold nanoparticles so ingested not only prevent disorders but demonstrate promising results such as boosting immunity, improving memory, and treating conditions such as respiratory disorders and autism. The study of the mode of action of Swarna Bindu offers to bridge the gap between traditional and modern medicine. The study explores the plausible intersection of modern nanoparticle therapy and traditional practices while emphasizing the advantages of gold nanoparticles in future clinical applications.

The impact of zinc on brain function, cellular dynamics, and its therapeutic potential in neuronal regeneration

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Zinc is an essential trace element in brain function, cell proliferation, and differentiation. It is the second most plentiful transition metal after iron in the central nervous system. In the central nervous system (CNS), zinc plays a crucial role in neurotransmission, synaptic plasticity, and neurogenesis, contributing to cognitive processes such as learning and memory. Maintaining zinc homeostasis is vital for neural health, as imbalances are linked to neurological disorders like Alzheimer's disease(AD), Parkinson's disease(PD), and Amyotrophic lateral sclerosis (ALS). Zinc regulates signalling pathways that control cellular growth, survival, and maturation, making it a key player in neuronal repair and regeneration. It promotes multipotent stem cells including mesenchymal stem cell proliferation and drives differentiation into specific neuronal lineages, supporting brain development. Zinc also acts as a signalling molecule in neurogenesis which can activate signalling pathways like the PI3K/Akt and MAPK pathways, which are key regulators of cell growth and proliferation. Its interaction with proteins involved in epigenetic modifications further highlights its role in cell fate determination. In the context of traumatic brain injury (TBI) and stroke, zinc plays a dual role, contributing to damage through excitotoxicity while also promoting healing by enhancing neurogenesis and neurite outgrowth. Experimental models of brain injury suggest that zinc supplementation could aid recovery by supporting axonal regeneration and synaptic connectivity. This review consolidates current knowledge on zinc’s role in brain function, its influence on cell proliferation and differentiation, and its therapeutic potential in neuronal repair. Understanding these molecular mechanisms may pave the way for zinc-based strategies to treat neurological disorders and injuries.

Dietary prebiotics as modulators of trace element homeostasis: A systematic review

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Prebiotics play a pivotal role in modulating the absorption of trace elements, influencing both beneficial and harmful elements bioavailability. Prebiotics, including inulin, oligofructose, beta-glucans, pectin, and fructo-oligosaccharides, modulate gut microbiota composition, improve gut barrier function, and chelate minerals, facilitating absorption. Conversely, prebiotics bind and sequester toxic trace elements, regulate metal transporter expression, and enhance gut motility, minimizing harmful element absorption. Studies demonstrate significant increases in beneficial trace element bioavailability, with prebiotic supplementation enhancing absorption of zinc, iron and copper; reduces toxic trace element absorption, with notable decreases in lead, mercury, and cadmium bioaccumulation. This comprehensive review highlights prebiotic's potential as a non-invasive, dietary intervention to optimize trace element balance, mitigating deficiencies and toxicity. Future research should focus on elucidating optimal prebiotic dosing regimens, oligosaccharides combinations, and probiotic synergies to maximize trace element absorption benefits, supporting evidence-based strategies for promoting public health and preventing trace element-related disorders.

Bacterial lipase activity could be altered by incorporation of bivalent cations

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Microbial lipases, in particular bacterial origin, are increasingly valuable due to their involvement in a wide range of chemical reactions. Lipases are a family of enzymes that break down triglycerides into free fatty acids and glycerol. The present study aimed at identifying probiotic bacterial strains possessing lipase activity and influence of trace element co-factors. Based on *in silico* screening, a total of 32 potential lipase producing probiotics have been identified. Qualitative and quantitative screening for their lipase production was carried out with three different oil sources at different incubation periods. Based on their lipase activity the organisms were further classified as high, medium and low/non-lipase producers. To assess the influence of co-factors (bivalent cations), five cultures (three highest and two least) lipase producing probiotics were investigated. The cations like iron, zinc, copper, calcium, magnesium, manganese and chelating agent EDTA (negative control) were added to the growth media @ 0.02%. The media without the addition of cations or EDTA served as control and all the cultures were incubated and the lipase activities were quantified titrimetrically. Evidently, the addition of different bivalent cations influenced the lipase activity differently. Incorporation of minerals had higher influence on poor lipase producing isolates (*Bacillus coagulans* SB-1 and *Bacillus subtilis* RG14) than high producers (*L. rhamnosus* 2021; *L. fermentum* 9338; *L. fermentum* LS-MS-1). The presence of EDTA negatively affected the lipase activity among the experimental cultures (-8 to -20%). Among the minerals, calcium (10 to 290%) and iron (4 to 300%) had positive impact, while effect of magnesium, manganese and zinc were low/nil. The presence of copper in media had strong negative (-10 to -34%) effect on lactobacillus; nevertheless, it potentiated lipase production in Bacillus isolates (500 to 1000%). It could be concluded that the lipase activity among different classes of probiotics could be influenced by bivalent cations, but studies with varying pH on mineral availability will provide more insight on their role in lipase activity.

Maternal supplementation of organic zinc improved offspring's gut health in broiler chickens

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A healthy gut is essential for efficient conversion of feed into its basic components for optimal nutrient absorption. The intestinal villus and crypt morphology in chickens has been associated with intestine function and chicken growth. Zinc, an essential trace element in growth, healing, and multiple cellular functions. An investigation on the effect of feeding organic zinc (zinc – methionine) to broiler breeder on progeny's intestinal development and performance was undertaken. A total of 100 adult (about 35 weeks of age) broiler breeders (CARIRO VISHAL), 80 hens and 20 males were divided into four treatments with four replicates each. The birds were fed standard breeder's diets as 0 (control with 40ppm Zn), three supplementary levels of zinc-methionine (Zn-Me) 20, 40 and 60 ppm for eight weeks. Subsequently, the hens were inseminated with semen from males of respective feeding regimen. The group-wise fertile eggs were collected, hatched and the chicks were reared separately as per maternal diet for 42 days. The bird's body weight, intestinal length and histo-morphology (villi length, width, crypt length and mucosal layer thickness) were studied. The body weight and intestinal lengths were similar ($P>0.05$) among the groups. Intestinal morphological attributes were significantly ($P<0.05$) improved in the supplementary groups as T3 (60 ppm) recorded higher villi length (1106.63 µm) and diameter (853.82 µm) followed by T1 (954.34 µm) and T2 (932.54 µm). From this study, it is concluded that the supplementation of organic zinc (60 ppm as Zinc-Methionine) to the maternal diet improved offspring's intestinal development.

Dietary chromium supplementation in young dairy calves reduces the risk of calf diarrhea by ameliorating insulin response, antioxidant status and immune response

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The aim of this study was to determine the effect of Cr supplementation on insulin sensitivity, lactose intolerance, diarrhea, antioxidant, and immune response in young Hariana calves. A total of 24 Hariana calves were randomly allocated into four groups having six calves in each group and fed for a period of 90 days. Experimental calves either received a basal diet devoid of supplemental Cr ($Cr_{0.0}$ group) or were supplemented with 0.05 mg ($Cr_{0.05}$ group), 0.10 mg ($Cr_{0.10}$ group) and 0.15 mg ($Cr_{0.15}$ group) of Cr/kg $BW^{0.75}$ as Cr- picolinate. Experimental calves were monitored fortnightly for body weight change. A more rapid glucose disappearance with unaltered insulin kinetics during intravenous glucose tolerance test (IVGTT) indicates greater insulin sensitivity in Cr supplemented calves. Better insulin sensitivity in Cr supplemented calves was further confirmed by higher values of the quantitative insulin sensitivity check index (QUICKI), revised quantitative insulin sensitivity check index (RQUICKI) and insulin receptor substrate-1 (IRS-1) and lower ($P<0.05$) values of homeostasis model assessment-insulin resistance (HOMA-IR) and glucose-to-insulin ratio in $Cr_{0.10}$ and $Cr_{0.15}$ groups during IVGTT. Cr supplementation resulted in a lower ($P<0.05$) serum cortisol concentration, whereas serum non-esterified fatty acid (NEFA) concentrations during IVGTT did not differ among the groups. No treatment differences were detected in the biomarkers of antioxidant status and immunity. As the age of the calf advanced, plasma concentration of glucose and insulin decreased ($P<0.05$) while the concentration of IRS-1 and IGF-1 increased ($P>0.05$). The incidence, duration of diarrhea, and fecal score were better ($P<0.05$) in calves fed on Cr supplemented diet whereas, no treatment effect was observed on average daily gain (ADG). Feeding a Cr-supplemented diet improved insulin sensitivity and reduced the risk of diarrhea in milk-fed young calves, but had no or minimal effects on antioxidant status, immune response, and growth performance.

Synthesis of novel selenocompounds as promising bactericidal compounds

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PURPOSE: It is estimated that 700,000 deaths are annually caused by antimicrobial resistance (*Life (Basel). 2022;12(4):468*). Therefore, there is an urgent necessity to develop new treatments for bacterial infections.

Selenium plays a critical role in the composition of biological molecules in both bacteria and eukaryotes (*Biochim Biophys Acta 2009;1790(11):1424-8*). Several selenocompounds have been reported to have antibacterial properties, including selenoureas, selenoanhydrides, selenocyanates, seleneoesters and diselenides (*Antibiotics (Basel). 2021;10(3):321*) (*Molecules. 2019;24(8):1487*) (*Microbiol. Res. 2013, 168, 563–568*) (*Bioorg. Chem. 2018, 79, 319–322*) (*Molecules. 2022;27(21):7477*). For this purpose, and giving the properties of Se, Se-containing molecules could be possible alternatives in the development of a new approach to combat infections.

METHODS: Several derivatives have been designed using a fragment-based approach and synthesized. These derivatives present both imine and Se in the form of selenocyanate and diselenide, which have been reported as antibacterial agents. The *in vitro* inhibitory activity of these compounds has been evaluated against *E. coli* and *S. aureus* bacteria strains by using a micro-broth dilution method.

RESULTS: According to the obtained results, selenocyanate derivatives showed better inhibitory activity than their diselenides analogs against both strains, highlighting those compounds containing a fluor or nitro substituent in the structure, with Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) values of 25 μg/mL. Interestingly, none of the diselenide compounds presented inhibitory activity against the Gram-negative bacteria, whereas some diselenides presented moderate inhibitory activity against the Gram-positive bacteria.

CONCLUSIONS: Bearing in mind the obtained results, merging in the same structure the imine and selenium moieties could be considered as an interesting starting point to obtain novel selenoderivatives with bactericidal activity. Thus, the results obtained are promising and further studies are needed.

The impact of sodium selenite and seleno-L-methionine on stress erythropoiesis in a murine model of hemolytic anemia

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Selenium (Se) is an essential trace element that exerts most of its biological activities through selenoproteins. Literature suggests the importance of dietary selenium (Se) as a key regulator of red cell homeostasis, and stress erythropoiesis. However, the comparative impact of organic and inorganic forms of Se in the diet on stress erythropoiesis in the spleen and anemia recovery are not known. Here we examined if inorganic (sodium selenite; Na_2SeO_3) or organic (seleno-L-methionine, Se-Met) forms of Se within different concentrations (deficient, adequate, supplemented, and supranutritional) support stress erythropoiesis and assist in the recovery from anemia. Three-week-old male C57BL/6 mice were subjected to graded levels of Se in the form of <0.01 ppm Se (Se-deficiency; Se-D), 0.1ppm Na_2SeO_3 (adequacy), 0.4ppm Na_2SeO_3 (supplemented), 3ppm Na_2SeO_3 (supranutritional), 0.4ppm Se-Met (supplemented), or 3ppm Se-Met (supranutritional), for 10-12 weeks prior to intra-peritoneal phenylhydrazine (PHZ) administration to induce hemolytic anemia. Following three days post-PHZ injection, spleen and blood samples were used to assess the impact of form and graded levels of Se in the diet on stress erythropoiesis. Phenotypic parameters showed that supplementation of the diet in Se with Na_2SeO_3 or Se-Met effectively alleviated the severity of hemolytic anemia. Compared to Se-D, Se in the form of adequate, or supranational Se as either Se-Met or Na_2SeO_3, supported the formation of multicellular complexes of macrophages and erythroblasts, as erythroblastic islands (EBIs), to efficiently promote stress erythropoiesis. Interestingly, 0.4 ppm supplemented Se-Met enhanced the differentiation of stress erythroid progenitors during anemia, while 0.4 ppm supplemented and 3 ppm supranutritional Na_2SeO_3 impacted the recruitment of monocytes and accelerated their differentiation into macrophages, to support their function within the EBI. Notably, 3-ppm supranutritional Se-Met elicited a more robust inflammatory response than an equivalent dose of Na_2SeO_3. While both Se-Met and Na_2SeO_3 effectively aided in anemia recovery, Na_2SeO_3 supplementation effectively supported stress erythropoiesis with a minimal inflammatory response, while Se-Met at supranutritional dosage led to increased inflammation despite its support for stress erythropoiesis. These results indicate diverse mechanisms of action of Se on the alleviation of anemia by stress erythropoiesis, which should be considered for further studies to complement existing therapies.

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Cymbopogon citratus-inspired zinc oxide nanoparticles: Synthesis, characterization and its biological assessment

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The study investigates the synthesis and biological applications of zinc oxide nanoparticles (ZnO NPs) produced through greener approach using *Cymbopogon citratus* leaf extract. The aim was to evaluate physico-chemical properties of the nanoparticles and their biological activities, including antioxidant, anti-inflammatory and their potential effects against pathogenic microbes *in vitro*. The green synthesized ZnO NPs were characterized by various analytical techniques. Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM) measurements revealed an average particle size of approximately 8.7 nm and 8.23 nm respectively, with a spherical morphology. Ultraviolet-visible (UV-Vis) spectroscopy confirmed ZnO with an absorption peak at 391 nm. X-ray Diffraction (XRD) analysis indicated a crystalline structure consistent with the wurtzite hexagonal phase and a crystalline size of 5 nm. Fourier Transform Infrared Spectroscopy (FTIR) identified Zn-O bonds and several other phytochemicals from the leaf extract. Biological assessments demonstrated the antimicrobial properties of the ZnO NPs using the well diffusion method. ZnO NPs exhibited effective concentration-dependent inhibition against pathogenic strains of *Escherichia coli* and *Salmonella* spp. with a minimum inhibitory concentration of 1000 µg/ml. Conversely, the nanoparticles showed no significant activity against *Staphylococcus* spp. The antioxidant activity assessed using the DPPH assay revealed a pro-oxidant effect at all concentrations tested (50, 100, 200, 300, 500 and 1000µg/ml). Additionally, the ZnO NPs exhibited dose-dependent anti-inflammatory activity in membrane stabilization assay, providing significant protection against red blood cell hemolysis. In conclusion, green-synthesized ZnO NPs exhibited effective antimicrobial (except against *Staphylococcus* spp.) and anti-inflammatory properties, despite having pro-oxidant tendencies. These findings suggest promising applications in various industries, including pharma, animal feed and textiles, though further research is needed to fully understand their practical applications and limitations.

Assessment of zinc bioavailability from bio-fortified rice: An *in vivo* rat study

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Through various breeding approaches, the micronutrients (zinc, iron, vitamin A, folates) content has been enriched in bio-fortified crop varieties for achieving the nutritional security. The present study assessed the bioavailability of zinc from bio-fortified rice in comparison to conventional rice in Wistar rats. The experimental ration was formulated using conventional (MTU 1010) and Zn bio-fortified (DRR Dhan-48) rice and pellets were prepared. The Zn content in conventional and bio-fortified rice were 14 and 24 ppm, respectively. Forty weaned Wistar rats were randomly allotted to two groups' *viz*., conventional rice (control) and bio-fortified rice group (bio-fortified) of 20 rats each with equal number of male and females. Rats were fed with experimental diets for 7 days of the initial adaptation period and experiments were carried out for 45 days. The feed intake was recorded daily, body weight was recorded at weekly intervals and digestion trial was conducted for 5 days at the end of the trial. At the end of feeding experiment, the rats were sacrificed to collect the vital organs to assess the distribution of minerals along with blood plasma. The mean body weight at the end of experiment was comparable among the groups (246 and 240 g) with similar food consumption. The g food intake per g gain was also comparable (3.30 and 3.16) (P=0.126). Zn absorption from bio-fortified rice was 45.12% as compared to 26.65% in conventional rice. The biologically available zinc from bio-fortified rice was 230% as compared to conventional rice (100%). The zinc content in different tissues (liver, bone, muscle, intestine and plasma) were similar between the two groups. The results indicated that zinc from bio-fortified rice has higher bioavailability, but their distribution/excretion pattern needs to be studied.

Boron as additive protects sperm motility and mitochondrial membrane potential during cryopreservation of Jersey semen

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The impact of boron (B), a vital element essential for various biological processes has not been explored for improving reproductive health of the mammals. Earlier study from this laboratory revealed positive impact of B on sperm output and motility, in the present study, protective effect of B as additive in the Jersey semen extender has been explored. The Jersey semen samples (n=8) were collected using artificial vagina and diluted in Tris-based extender (80 million sperm /ml). The diluted semen samples were divided into four groups, control and three boron-treated groups (0.3, 0.45, and 0.6 µg/100 µl) and filled in 250µl French mini straws and subsequently cryopreserved in liquid nitrogen as per the established protocol. After one week of cryopreservation, the semen samples were thawed at 37°C for 30 sec and subjected to functional parameter analysis. The sperm kinematics were analysed using computer assisted semen analyzer. The sperm mitochondrial membrane potential (MMP) and reactive oxygen species (ROS) were assessed using JC-1 and DHR 123 dyes, respectively employing flow cytometry. B at 0.45 µg/100 µl in semen extender significantly enhanced sperm total motility ($p<0.01$) and MMP ($p<0.03$) as compared to control group. The ROS level increased significantly at higher doses of B (0.45 and 0.6 µg/100 µl) as compared to the control group. The kinematic parameters, including curvilinear velocity, average path velocity, and straight-line velocity did not differ significantly between control and boron-treated groups. The study suggests that B at a dose of 0.45 µg/100 µl during cryopreservation is effective for improving sperm motility and mitochondrial membrane potential in Jersey bulls. However, the effect of increase in ROS, which may be common due to elevated MMP, and field fertility rate need to be addressed to understand the effect of B as additives in the bull semen.

Impact of *Limosilactobacillus reuteri* and Jerusalem artichoke synbiotics on immune response and serology in Wistar rats

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This 42-day study investigates the potential of functional foods, specifically synbiotics, to modulate the immune response in Wistar rats both independently and following antibiotic treatment. A total of 48 Wistar rats, aged 3-4 weeks with an average body weight of 89±4 g, were selected based on fecal microbiome antibiotic sensitivity, revealing ciprofloxacin (CIP) as the most effective and ampicillin (AMP) as the least effective antibiotic. The rats were divided based on body weights into six treatment groups: Control (basal diet), SYN (synbiotics), AMP, AMP+S (ampicillin plus synbiotics), CIP, and CIP+S (ciprofloxacin plus synbiotics). The synbiotic regimen included *Limosilactobacillus reuteri* RM125MT903467 (10 $^{8\text{-}9}$*CFU/Ml)* and JAT powder@2% of DM intake. Following 14 days of antibiotic administration, synbiotics were introduced to the AMP+S and CIP+S groups.

To evaluate the immune response, delayed-type hypersensitivity (DTH) was assessed by subcutaneously injecting phytohaemagglutinin-P (PHA-P) into all rats. The immune response was quantified by measuring the percentage increase in skin thickness at the injection site at 12-, 24-, 48-, and 72-hours post-injection, relative to baseline measurements. The relative changes in skin thickness among the treatment groups ranged from 100% to 248% . The AMP group exhibited the highest DTH response at 24 hours ($P<0.05$), followed closely by the CIP group, while the control group showed the least response. Serological analyses indicated no significant differences ($P>0.05$) in liver and renal function indices or lipid profiles across treatment groups. These findings suggest that the administration of synbiotics, either alone or following a period of antibiotic treatment, enhances the DTH immune response in rats. Notably, the use of antibiotics, particularly ampicillin and ciprofloxacin, significantly heightened immune responses compared to the control group. This study underscores the potential of synbiotics to bolster immune function in the context of antibiotic use.

Organic and nano trace minerals at reduced dietary levels sustains the growth performance and nutrient metabolizability in broiler chicken

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A study was conducted to evaluate the effect of dietary supplementation of organic and nano trace minerals at reduced levels on the growth performance and nutrient metabolizability in broiler chickens. 240 day-old commercial broiler (Cobb strain) chicks were assigned in equal numbers to five groups consisting of four replicates each. The basal diet (T1) was supplemented with inorganic trace minerals at 100% of the standard recommendation to serve as control (ITM 100). Test diets were prepared by supplementing organic trace minerals at 75 (OTM 75) and 50 % (OTM 50) of the standard recommendation or nano trace minerals at 50 (NTM 50) and 25% (NTM 25) of the standard recommendation. Each diet prepared for pre-starter (1-7 days), starter (8-21 days) and finisher (22-42 days) phases was offered to respective groups. The results revealed that significantly different ($P<0.05$) body weights of the birds were observed at the end of 1st, 2nd and 3rd week while no such difference were observed at the end of 4th, 5th and 6th week. There was no significant difference ($P>0.05$) in weekly cumulative feed intake and feed conversion ratio throughout the experimental period. A metabolism trial during last week of the trial revealed that the metabolizability of proximate nutrients in broiler diets supplemented with organic and nano trace minerals at reduced levels were comparable with that of control diet (ITM 100). It was concluded that similar growth performance and nutrient metabolizability can be achieved by replacing inorganic trace minerals (Mn, Cu and Zn) with organic and nanoparticles trace minerals at 50 % and 25 % of the standard recommendation, respectively in broiler diets.

Influence of boron supplementation on mineral profile in sheep fed diets with or without adequate levels of calcium

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The present study was planned to investigate the influence of dietary boron (B) on deposition of boron, bone forming minerals (Ca, P, Mg), other trace elements (Cu, Zn, Mn) in rib bone, vital organs (liver, kidney) and carcass characteristics. A feeding trial for180 days was carried out with twenty four Bandur crossbred ram lambs (5-6 months) arranged in a 2x2 factorial design, fed on total mixed ration (TMR: 60 roughages, 40 concentrate) with two levels each of boron (0 or 40 ppm) and calcium (100 or 50% of requirements), were randomly divided into 4 dietary groups (6 in each) viz., Normal Calcium (Control), Low Calcium (Ca-50%), Normal Calcium with B (40 ppm) and Low Calcium with B (Ca-50% + 40 ppm B). Five sheep from each group were sacrificed to collect rib bone, liver, kidney and study carcass parameters. Irrespective of Ca level in rations, concentrations of Ca, P, Mg, Cu and B in rib bone were significantly ($P<0.05$) increased with B-supplementation in lambs. Supplementation of B did not significantly alter the content of minerals (P, Mg, Zn, Cu and Mn) in liver. The Ca content in liver was significantly ($P<0.05$) higher in Ca-adequate ration supplemented with B. In kidney, mineral profile (Ca, P, Mg, Zn, and Cu) remained unaltered except for Mn content which was significantly ($P<0.01$) decreased in kidney of lambs fed on dietary boron. Supplementation of B did not show any effect on carcass characteristics of lambs except improving the weight of edible organs like spleen and kidney. The present findings indicate the positive effect of dietary boron in improving the deposition of bone forming minerals (Ca, P, Mg) and other trace elements (Cu, B) in sheep.

Posters

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Evaluation of selenium functionalized nano composite in the cellular models of GBM

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Glioblastoma multiforme is the most aggressive malignant brain tumour. Limitations associated with conventional treatment necessitate the development of alternative approaches for radiotherapy adjuvant drugs. Gold (Au) nano-conjugates and diselenides (-Se-Se-) can exhibit anti-cancer effects through redox modulation. Therefore, the project's goals were to demonstrate radiosensitization in GBM cells and optimize the process for creating peptide-diselenide gold nanoparticles (Au@G-Se-Se-G), using gelatin, an FDA approved excipient. Size and shape selective selenium functionalised gold nanocomposites were synthesised and optimised. The irradiation of cells was performed using a ^{60}Co Bhabhatron γ-source (Department of Atomic Energy, India) with a dose rate of 0.34Gy / min. all the cellular experiments were carried out according to the reported methodologies. Diselenide functionalized gelatin (G-Se-Se-G) was prepared by covalently conjugating gelatin with diselenodipropionic acid through EDC/NHS coupling chemistry and subsequently used to replace citrate from AuNP surface, obtaining Au@G-Se-Se-G NPs. NPs were of spherical morphology with an average size of about 15 nm and were characterized in detail by TEM, EDS, Raman spectra, DLS, FTIR, CV, and other techniques. Later, Au@G-Se-Se-G's radiosensitizing effect on LN229 cells were investigated in GBM cells, and the findings showed that combination of radiation with the drug lead to a significant reduction in survival fraction and exhibited necroptotic mode of cell death. Together, the above results establish selenium functionalization of gold nanoparticles as an effective strategy to achieve radio-sensitization in the cellular models of GBM.

Gut bugs-great benefits: Probiotics and trace elements

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Probiotics are beneficial live microorganisms especially bacteria and yeast when ingested in adequate amount confer various health benefits. They have gained significant attention for their role in gut health, immune function and mental health promotion. Emerging studies enlighten that probiotics may also play a critical role in enhancing the bioavailability of essential trace elements in the human body. Key trace elements like iron, zinc, and magnesium are well known for its vital role in various physiological processes, including immune function, cognitive development and bone health. Probiotics influence absorption of trace elements through different mechanisms. They can regulate the gut microbiome, reducing inflammation and creating favorable environment for nutrient absorption. Probiotics directly bind and transport essential metals, enhancing their bioavailability. Individuals may optimize their trace metal status and overall health by incorporating probiotic rich foods or supplements and beverages into a balanced diet. Further research is required to fully elucidate the complete mechanisms underlying this relationship and identify the specific potent probiotic strains.

Trace elements: The key players in bone regeneration

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Trace elements, and essential micronutrients play a crucial role in bone health. These elements including copper, zinc, selenium and others influence diverse aspects of bone metabolism, such as cell differentiation, cell proliferation, and apoptosis. By modulating the key signaling pathways such as BMP-2, TGF-β1/Smad and Wnt/β-catenin, trace elements regulate bone re pair and forma5on. Moreover, they act as potent antioxidants, which help protect cells from oxidative stress-induced damage. Researchers are exploring various strategies to utilize the potential of trace elements in bone regeneration. Dietary supplementation can guarantee adequate intake of these micronutrients. Direct integration of trace elements into biomaterials can accelerate the healing of bone defects. In specific case s, systemic administration of supplements may be beneficial. Further studies in this path should focus on developing innovative delivery systems such as nanotechnology- based carriers, to improve the bioavailability and targeted delivery of trace elements to the bone 5ssues. By strategically combining element supplementation with other therapeutic interventions, like growth factors or stem cell therapy synergistic effects can be achieved.

Evaluation of aantibacterial activity of molybdenum doped cerium oxide nanoparticle

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Trace elements such as cerium and molybdenum contribute to catalysis, which can be used in antimicrobial applications. Cerium oxide NPs are used in different research fields due to their distinctive characteristics. The incorporation of molybdenum in nanostructures like cerium oxide can provide insights into its biological activity and possible impact on cellular processes. Mo-CeO_2 NPs may mimic natural enzymatic activity, producing reactive oxygen species (ROS), which restrict bacterial growth. This is especially relevant when considering how trace elements can be used to impact microbial ecosystems in the body. In this work the cerium oxide NPs were synthesized by solution combustion method and subsequently doped with molybdenum with different concentration (X=0.01, 0.02, 0.03, 0.04, 0.05). The formation of NPs was confirmed by X ray diffraction (XRD), UV-Visible absorption, Fourier-transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM). The antibacterial properties of the CeO_2 and doped CeO_2 NPs against Gram- positive (*S. aureus*) and Gram- negative (*P. aeruginosa*) bacterial strains were explored. The findings reveal an increased efficacy in antibacterial activity of cerium oxide NPs doped with molybdenum.

Keywords: Cerium oxide, Molybdenum, Antibacterial activity, *S. aureus*, *P. aeruginosa*

Phytoferritin from plant derived nanovesicles as a novel bioavailable source of iron to treat anemia

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Isolation, characterization, *in vitro* and *in vivo* bioavailability of the plant derived nanovesicles containing phytoferritin as the bioavailable source of iron. The plant derived nanovesicles (PDNVs) from fenugreek seeds, soyabean, cumin, fennel, spinach, mint, moringa and red sorrel were isolated by gold standard ultracentrifugation method. The size distribution and transmembrane negative potential of the PDNVs was determined by size and zeta potential analyser and size was confirmed by scanning electron microscopy (SEM). The presence of lipids was determined by thin layer chromatography (TLC). PDNVs were screened for phytoferritin by western blot using plant ferritin specific antibody, where partially enriched pea ferritin (PF) was used as positive control. The intracellular delivery of phytoferritin was confirmed *in vitro* by immunocytochemistry (ICC) and the gastro-intestinal stability was determined by *in vitro* digestion. *In vivo* bioavailability of ferritin-iron complex in PDNVs and toxicity assessment was performed. The size of the PDNVs isolated from seeds ranged from 175.9 nm-361.0 nm, while those of green leafy vegetables ranged from 379.1 nm-453.9 nm. SEM photographs exhibited the presence of nano-sized vesicles and TLC validated presence of lipids in PDNVs. In initial screen with different PDNVs, we specifically detected phytoferritin in fenugreek seed-derived nanovesicles (FGDNVs). Phytoferritin in FGDNVs were present in its native nanocage form containing iron and was resistant to proteinase digestion. Unlike pea ferritin (PF), FGDNV ferritin was stable in simulated *in vitro* digestion and delivered ferritin in mammalian cells. In IDA model *in vivo*, FGDNVs containing mere one tenth of recommended dietary allowance of iron, rescued iron deficiency and restored hematological parameters due to its enhanced bioavailability compared to PF in gastrointestinal tract. Thus, FGDNVs represent a natural iron nano-formulation for safe and efficient therapeutics for IDA.

Nano-calcium and aqueous extract of *Tagetes erecta*-based hemostatic powder for topical applications

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The present study focuses on development of a polyherbal based haemostatic formulation containing polyherbal active, nano-calcium, psyllium and xanthan gum. Nano calcium was prepared using probe sonication method and analysed for PDI and particle size. Herbal actives were screened using milk coagulation assay and in-vitro blood clotting time. Based on the obtained results, aqueous extract of *Tagetes erecta* was selected for further study. Formulation was developed and evaluated for in-vitro, in-silico and in-vivo haemostatic properties. Nano-calcium and aqueous extract of *Tagetes erecta* based haemostatic powder for topical applications. Marigold leaves were shade dried and aqueous decoction was prepared. Nano-calcium was and screened for milk coagulation and *in-vitro* blood clotting time. Prepared nano-calcium was observed to have PDI of 0.293 and average particle size was found to be 396.7nm. Among various actives subjected for milk coagulation assay and *in-vitro* blood clotting time, aqueous extract of *Tagetes erecta* showed 40 sec and 90 sec results and hence was selected to incorporate into formulation. Formulation containing aqueous extract of *Tagetes erecta* showed good platelet aggregation and blood clotting index was found to be 26.04. Docking score was found to be -6.66 for kaempferitrin compound. *In-vivo* blood clotting time was found to be 75 sec for formulation compared to 160 sec for marketed product and 265 sec for control. Overall, the study findings have suggested that formulation containing nano-calcium and aqueous extract of *Tagetes erecta* can be successfully used for topical acute haemostatic conditions.

Selenium and immune checkpoint inhibition in acute myeloid leukemia

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Acute myeloid leukemia (AML) often relapses because leukemia-initiating stem cells (LICs) are resistant to currently existing therapies. Earlier research from our laboratory reported that LICs from AML patients and a murine AML model were sensitive to selenium (Se)-dependent mechanisms involving endogenous metabolites such as prostaglandin D_2 metabolites, Δ^{12}-PGJ_2 and 15d-PGJ_2, collectively called cyclopentenone prostaglandins (CyPGs). When the AML mice were supplemented with 0.4 ppm Se (as sodium selenite), a corresponding increase in CyPGs was associated with increased remission and survival. These effects were mediated through Gpr44, a G-protein coupled receptor, on the LICs. Further studies indicated increased expression of programmed death ligand 1 (PD-L1), an immune checkpoint protein, in $Gpr44^{-/-}$ LICs. PD-L1 binds to its receptor, PD1, on T-cells to inhibit anti-tumor immune mechanisms leading to poor disease prognosis. Flow cytometry-based analysis in AML mice maintained on graded Se diets, transplanted with WT or $Gpr44^{-/-}$ LICs, indicated a direct correlation between increased dietary Se intake and Gpr44 activation in both LICs and T-cells to reduce the expression of PD-L1 and PD1, respectively. Such a significant reduction in PD-L1 and PD-1 expression correlated with increased Th1 cytokine profile suggesting the release of “immune checkpoint brake(s)” leading to efficient immune surveillance and control. These findings highlight the many factors involved in AML progression and offer new strategies for potential therapies to treat AML successfully.

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Impact of protein and micronutrient blend on the characteristics of cookies made with whole wheat flour

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In the present study protein and micronutrient premix used to develop functional atta (whole wheat flour) for cookies.

Atta was replaced with 10% soy protein isolate (SPI), soy protein concentrate (SPC), defatted soy flour (DSF), whey protein concentrate (WPC), whey protein isolate (WPI) and 5% whole egg powder (WEP), egg yolk powder (EYP) and egg albumin powder (EAP) with 25% RDA of micronutrients such as zinc sulfate, calcium carbonate, and sodium iron EDTA to study their effect on physicochemical, nutritional, textural, and sensory properties of cookies.

The results revealed that the addition of an increasing amount of protein source in blends with mineral premix, highest L value 82.71 and lowest was 79.54 among all blends and highest protein content was SPI (19.94%) lowest in DSF (14.93%). The ash content was highest in DSF (1.86%) and lowest in SPC (1.69%). Mineral analysis showed highest zinc content 9.84mg in SPC blend compared to control and calcium and iron was highest 395.79 (WPI 10%) and 24.988 mg/100g in DSF 10% blend with mineral premix. The diameter and spread ratio were highest in WPC and WPI 5.75mm and 4.49 w/t and least was SPC 5.18mm and DSF 1.19w/t. The decrease in spread ratio could be due to dilution and disruption of gluten strands by adding protein, thereby losing its elastic recovery properties. The hardness of cookies was found highest in EAP blend (198.11N) and least hardness was seen in EYP (69.77N). Sensory evaluation showed that incorporating protein and micronutrients premix of cookies had clean mouthfeel without any residue formation and the acceptable cookies with all the blends. The highest overall quality of cookies was found in DSF (6.4) and lowest was EYP (5.1).

Punica granatum derived Nano ZnO: DNA protection from oxidative damage and cytotoxic effect on cancer cells

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Zinc (Zn) is a vital trace element in the body, serving as an essential catalytic, structural, and regulatory ion. Zinc interacts with oxygen to produce zinc oxide (ZnO), which can be used in wide variety of applications, much of current industry as well as in biomedical fields .However, with the advent of nanotechnology it is possible to explore new properties of ZnO at nanoscale dimension which has led to a renewed interest in this material. Thus, ZnO in nanoscale has shown antimicrobial properties and potential applications in food preservation. ZnO is a bio-safe material that possesses photo-oxidizing and photo catalyst impacts on chemical and biological species. Recently, ZnO nanoparticles have been incorporated in polymeric matrices in order to provide antimicrobial activity to the packaging material and improve packaging properties. The interaction of nanoparticles with microorganisms and bio molecules is an expanding area of research, which is still largely unexplored yet. In this study, ZnO nanoparticles have been synthesized via green synthesis route using pomegranate (*Punica granatum*) peel extract. The synthesized particles were black in colour which is very unique to the usual white coloured ZnO nanoparticles obtained through the green synthesis route. The ZnO nanoparticles synthesized had hexagonal morphology with average size range of 90-120 nm and a hexagonal wurtzite crystal lattice structure. Biosynthesized ZnO nanoparticles showed significant anticancer activity on HeLa cell lines (IC50 = 68.22 µg/ml) compared to MCF-7 cell lines (IC50 = 184.55 µg/ml). In addition to this, highly significant DNA protection activity was observed at a concentration of 1 mg/100µl of ZnO nanoparticles.

Intracellular formation of submicrometer-sized spherical elemental selenium by a novel selenite-reducing bacterium isolated from seleniferous soil

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Selenium is an essential trace element for many organisms but is toxic in excess amounts. Some microbes reduce water-soluble, toxic selenite to form less toxic, insoluble spherical elemental selenium (sSe^0), impacting the environmental selenium cycle. The size and properties of the sSe^0 produced vary greatly depending on the type of bacteria, with many aspects of the production process remaining unknown. Previously, we isolated a novel bacterial strain, *Cellulomonas* sp. D3a, from seleniferous soils in Punjab, India. This strain exhibits higher tolerance to selenite than closely related species and accumulates submicrometer-sized large sSe^0 intracellularly. Here, we investigated the process of sSe^0 formation in this bacterium. First, strain D3a was cultured with various concentrations of selenite under anaerobic conditions, and then the amount of remaining selenite and the amount of Se^0 formed during growth were quantified. The addition of selenite inhibited the growth in a concentration-dependent manner but rescued the cell death in the stationary phase. Selenite reduction and Se^0 formation were observed during the exponential growth phase, but not during the stationary phase. The process of sSe^0 formation within the cells was then analyzed using transmission electron microscopy and energy-dispersive X-ray spectroscopy. Surprisingly, non-spherical, irregularly shaped selenium-containing deposits were observed in the exponentially growing cells. However, the fraction of sSe^0 was increased during the long-time cultivation in the stationary phase. These results suggest that intracellular sSe^0 formation is mediated by the maturation of non-spherical Se^0 deposits after selenite reduction.

Mass Spectrometry in Proteomics: Current Applications and Future Directions

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Mass spectrometry (MS) has significantly transformed proteomics, offering deep insights into protein structure, functionality, and interactions. This review explores the present applications of mass spectrometry within the field, focusing on its contributions to protein identification, quantification, and the study of post-translational modifications. We highlight recent advancements in MS technologies, such as tandem MS and high-resolution instruments, which have improved both sensitivity and precision. Furthermore, we discuss the synergy between mass spectrometry and bioinformatics, as well as its integration with other omics approaches, enabling more comprehensive proteomic investigations. Looking ahead, we consider emerging trends, including single-cell proteomics, innovative mass spectrometric methods, and the prospects for real-time monitoring of proteomic changes in clinical contexts. This review aims to provide a thorough overview of the influence of mass spectrometry on proteomics while suggesting future research directions to enhance its applications in biology and healthcare.

Toxicity of mercury in wastewater treatment

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Mercury, or Hg for short, is a ubiquitous ecological contaminant that exists all over the globe. It is commonly found in three distinct oxidation states: elemental (Hg^0), mercurous (Hg^1), and mercuric (Hg^{II}). Since mercury (II) is not biodegradable, even minute amounts of it in water can cause permanent harm to people (such as damage to the heart, lungs, and central nervous systems, as well as DNA mutations), animals, and life in the water. Mercury removal from aqueous solutions is therefore a pressing necessity of the present day. A rapid industrialization and urbanization are the root causes of what we call a - “toxic chain” in the atmosphere, which refers to the accumulation of harmful pollutants in our ecological system. In general, determining the levels of mercury toxicity in wastewater is essential for preserving the environment, protecting human health, and guaranteeing the efficiency of wastewater treatment procedures. A wide number of treatment strategies have been used to remediate mercury pollution in wastewater, such as - Adsorption, Microfiltration, Membrane Separation, Membrane Distillation, Ozonation, Nano-remediation, Phytoremediation and Bioremediation. All these techniques are subjective to the operating conditions and mechanisms of removal. The problem of mercury contamination in industrial and municipal wastewater is a considerable one. Past investigations have clarified the problem of mercury emissions to the environment, which has been shown to have substantial effects on the health of both individuals and vegetation. Adsorption, membrane separation, ion-exchange, and ozonation are among the treatment techniques typically used to treat mercury in water or wastewater. The emerging issue is now the recovery of mercury from the aqueous phase. It will vary from wastewater treatment plant to wastewater treatment plant to determine the best combination of technologies. The toxicity of mercury and its potential impacts on ecosystems and human health make mercury poisoning of wastewater a major environmental issue to be addressed at a root level.

Heavy Metal Bioremediation

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Bioremediation is an innovative strategy that utilizes microorganisms, particularly fungi and bacteria, to address environmental contaminants. This study examines the effectiveness of two fungal genera, *Aspergillus* and *Penicillium*, in mitigating the impacts of textile dye effluents and heavy metals significant pollutants that threaten aquatic ecosystems and human health. Textile dye effluents significantly increase the chemical oxygen demand (COD) in water bodies, negatively affecting the growth, development, and reproductive viability of aquatic life. Dyes like Malachite Green and Rose Bengal are particularly persistent and toxic, posing serious health risks, including oxidative stress, to humans. Moreover, heavy metals such as cadmium, copper, lead, nickel, and zinc have intensified due to industrial activities, further harming the environment and disrupting ecological balance. This research evaluates the biosorption capabilities of *Aspergillus* and *Penicillium* in a controlled setting, using Sabouraud's Dextrose Broth (SDB) enriched with the selected dyes. Biosorption involves the absorption of pollutants by biological materials, offering an effective means of wastewater treatment. The study employed spectroscopic and colorimetric analyses to quantify dye absorbance and assess the biosorption effectiveness. Results indicate that both the chosen fungi, penicillium and Aspergillus have displayed relatively equal levels of biosorption. However, this may vary based on physicochemical properties like temperature, potential of hydrogen (pH) and pressure. In conclusion, this study emphasizes the promising potential of fungal species, particularly *Penicillium*, as effective agents in bioremediation efforts. By harnessing these natural processes, it is possible to develop sustainable strategies to mitigate the environmental consequences of textile dyes and heavy metals, thereby protecting human health and aquatic ecosystems.

Multifunctional chelators: A promising strategy for metal-related neurodegenerative diseases

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The misfolding of proteins and ensuing conformational alterations are key hallmarks in most neurodegenerative diseases, eventually resulting in the generation of toxic protein oligomers. Both genetic mutations which drive protein aggregation, and physiological factors especially labile metal ions (Fe, Mn, Cu, Zn-collectively known as trace elements) contribute immensely. Brain biochemistry require these metal ions and their equilibrium is frequently compromised in several neurodegenerative conditions associated with proteinaceous aggregates detectable in patients. Recently, chelation therapy has become a novel treatment for metal-overload diseases. Chelators must penetrate the blood–brain barrier, and also they need an additional functionality besides metal binding. Desirable properties would include cell-type selectivity, as well modest iron binding affinity to sequester labile iron without perturbing homeostasis and targeting mitochondria. This review focuses on recent progress in pharmacological chelation therapy for Parkinson's disease, Alzheimer's Friedreichs ataxia and pantothenate kinase-associated neurodegeneration. We also discuss the advent of multitargeted iron chelators that could provide better disease modifying benefits with a favorable safety profile than existing therapies for treating neurodegenerative diseases.

Minerals associated sperm-expressed genes majorly regulate energy production process and motility

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During artificial breeding, the role of male and semen quality assumes greater significance as semen from one bull is used for inseminating thousands of cows. The semen production behavior and quality of the semen is influenced by various molecular functions mediated by minerals and associated biological process. Earlier studies, we investigated the composition of sperm-expressed genes and their role in fertilization process in buffalo. The present study aims to identify mineral associated sperm-expressed genes and their molecular function in buffalo fertilization events. Sperm-expressed genes identified from the total RNA sequenced samples from buffalo sperm (n=7) were subjected to functional annotation using Gorilla web server. Approximately 10% of the sperm-expressed genes (186 out of 1939 genes, >1 FPKM) were associated with minerals. These are majorly related to zinc, calcium, phosphorus, copper, iron and sodium. The top clusters associated with these genes were kinesin complex (17 genes), dynein (14 genes), proton-transporting V-type ATPase complex (7 genes). The biological process of these genes were: cellular component organization or biogenesis (78 genes; FDR=0.0130), organelle organization (64 genes, FDR=2.09e-05), microtubule-based process (38 genes, FDR=8.49e-13), etc. These findings suggest the role of minerals in sperm organelle organization and microtubule function. The pathways associated with these genes were thiamine metabolism (FDR=0.0374), synaptic vesicle cycle (FDR=0.0440), etc. Thiamine has a critical role in oxidative metabolism during spermatogenesis and sperm motility, whereas the synaptic vesicle cycle is involved in cell-cell communication during fertilization events. The results of this study provide valuable insights into the influence of nutrition for optimal sperm production and fertility. In future, information from these minerals associated sperm-expressed genes, male-specific targeted nutritional supplements can be developed for improving male fertility in buffalo.

Diseleno-albumin, a drug free therapeutic system induces apoptosis through mitochondrial derived reactive oxygen species in lung cancer cells

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As di-selenide compounds have gained a lot of attention in the redox therapeutic arena of cancer, here we aim to develop a native bioinspired diselenide functionalised therapeutic protein for lung cancer treatment. For this, we have chosen bovine serum albumin (BSA) as the host molecule and 3-3'-diselenodipropionic acid (DSePA) as the pharmacophore moiety. The conjugate of BSA and DSePA, henceforth referred as Se-Se-BSA were investigated in detail to evaluate its potential as therapeutic protein against lung cancer model. Se-Se-BSA was synthesized following EDC-NHS carbodiimide coupling of BSA with DSePA. The product was purified through dialysis and characterised by electrophoresis, MALDI-TOF-MS, AAS, CD, XPS, and other spectroscopic techniques. The antiproliferative activity of conjugate was studied in cellular (A549) model of lung cancer by MTT and clonogenic assays. The mechanism of action was established by monitoring the effect of Se-Se-BSA on the cellular redox status, ROS generation, and apoptotic pathways using standard biochemical and cell biology assays. The spectroscopic characterisations confirmed the conjugation of approximately ~5-7 DSePA molecules per BSA molecule. The cytotoxicity studies by involving cell lines of cancerous and non-cancerous origins indicated that Se-Se-BSA selectively inhibited the proliferation of cancerous cells. The cellular uptake studies by physically labelling Se-Se-BSA with curcumin and following its intracellular fluorescence confirmed that uptake efficiency of Se-Se-BSA was similar to that of native BSA. Finally, studies on the mechanism of action of Se-Se-BSA in the A549 (lung adenocarcinoma) cells revealed that it induced mitochondrial ROS generation followed by mitochondrial dysfunction, activation of caspases and apoptosis. Together, these results demonstrate a bio-inspired approach of exploring diselenide (-Se-Se-) grafted serum albumin as the potential drug free therapeutic for anticancer application.

Turn-On fluorescent probe detection for fluoride; in-vivo, in-vitro, and enzymatic studies

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Fluoride, the smallest common anion, has a unique role in biological systems due to its high charge density and dual-edge characteristics. Fluoride is a key trace anion in the human body, has an important function in human health, particularly in improving dental health and lowering the occurrence of dental caries. It interacts with both H^+/hydronium (by hydrolysis) and strongly with Ca^{2+} ions, which are necessary for skeletal and bone chemistry. However, excessive fluoride intake can have negative consequences, such as fluorosis (dental and skeletal fluorosis), and has been linked to a moderate decrease in children's I.Q. As a result, fluoride intake must be regulated and monitored. In recent developments, fluoride-triggered Si–O cleavage has gained attention as a mechanism for aqueous fluoride chemosensing. Among various fluoride probes, **Myco-F** stands out as a novel and cost-effective probe that enables highly sensitive F^- detection in a fully aqueous medium, offering the lowest detection limit reported so far. Extensive studies, including those in cellular and zebrafish models, have demonstrated its efficacy(1).Furthermore, Myco-F has shown remarkable stability in aquatic conditions, with significant distinctions between enzyme-catalyzed and uncatalyzed hydrolysis (2). Its applications include detecting enzyme activity in cell lysates and identifying positive "hit" candidates in high-throughput screening. Furthermore, Myco-F has been utilized successfully in quantitative kinetic tests, providing useful Michaelis-Menten parameters. These findings suggest that Myco-F is a viable tool for biological study as well as practical applications such as fluoride detection and enzyme activity tests.

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Chemical speciation and total metal contents of Fe, Cu, Ni, Zn, in edible bamboo shoots of Manipur, Northeast India

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Bamboo shoots, consumed fresh, dried, fried, or fermented, are a significant dietary component and delicacy in Southeast Asia. Limited studies on essential trace elements in bamboo shoots have shown notable Fe, Zn, Cu, and Mn concentrations. However, these studies have not explored the speciation or the specific chemical forms of these elements. The goals of this research included: (i) screening a representative population of bamboo shoots from the State of Manipur, Northeastern India for essential trace elements, (ii) identifying their chemical forms, and (iii) correlating the element concentrations with those potentially available from soil. Samples of five commonly consumed bamboo species and corresponding soils were collected from five locations in Manipur, Northeast India. Bamboo sap was extracted by pressing fresh shoots. Soil samples were analyzed for bioavailable metal fractions using acetate extraction at pH 5. Inductively coupled plasma mass spectrometry (ICP-MS) determined total trace element concentrations. Speciation analysis was conducted using hydrophilic interaction liquid chromatography (HILIC) with dual ICP-MS and electrospray MS/MS detection. Data were statistically analyzed for variability using ANOVA. Notable amounts of Fe (12-81 mg/kg), Zn (35-166 mg/kg), Cu (4-34 mg/kg), Mn (0.1-2.1 mg/kg), Rb (15-112 mg/kg), and Ni (1-24 mg/kg) were recorded, with metal species forming complexes with nicotianamine (NA), deoxymugineic acid (DMA), and histidine. The geographical location did not significantly impact trace element patterns or speciation. However, Ni and, to a lesser extent, Zn concentrations and speciation were influenced by the bamboo variety. The consumption of bamboo shoots contributes to the dietary intake of several elements essential for the human health. Fe, Zn and Cu are mobilised from soil and transported upwards bamboo shoots as NA and DMA complexes, for Ni this transport also takes place also in the form of histidine complexes.

Bio-fabrication of nanoceria using green extract and their biomedical applications

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This research focuses on the bio-fabrication of CeO2 nanoparticles using *Solanum nigrum* (SN) leaf extract. The nanoceria are synthesized by employing the leaf extract as a reducing and capping agent. The synthesized nanoceria are then subject to various characterization techniques. UV- Visible spectroscopy for the conformation of the nanoceria, Powder X-ray diffraction (XRD) analysis is conducted to determine the crystal structure and phase purity of the nanoparticles. Fourier transform infrared spectroscopy (FT-IR) is used to identify the functional groups present in the synthesized nanoparticles and the leaf extract. Additionally, antioxidant activity and anti-inflammatory activity tests are conducted to explore the potential application of synthesized nanoceria. The nanoparticles were tested against drug (Ibuprofen) and chemical (Ascorbic acid) to evaluate their potent activity as an anti - inflammatory agent and antioxidant agent, respectively. Overall, this study presents a comprehensive investigation of the bio-fabrication of nanoceria using *Solanum nigrum* leaf extract, including their characterization through XRD, UV -Visible and FT-IR analyses and the application of these nanoparticles in the field of medical research.

Development of antioxidant nanozymes with antiplatelet activity for prevention of thrombosis

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Thrombotic events, such as ischemic stroke, deep vein thrombosis, pulmonary embolism, and myocardial infarction, are significant contributors to global mortality. Thromboembolic conditions have been estimated to account for 1 in 4 deaths worldwide. Thrombosis, typically characterized by the formation of intravascular clots, is a pivotal factor underlying acute cardiovascular and thromboembolic disorders. Thrombocytes or platelets, the smallest blood cells in our body play a dual role in regulating both prothrombotic and antithrombotic events. It is reported that platelet-dependent thrombus formation may be influenced by the alteration of platelet or vascular redox state, the presence of endogenous or exogenous antioxidants, and the formation of reactive oxygen and nitrogen species.

Antiplatelet therapy has been widely used for the prevention and management of thrombosis. Antiplatelet drugs limit the ability of platelets to form aggregates and prevent clot and thrombus formation thereby acting as a good measure to fight against thrombosis related diseases.

Nanomaterials, especially nanozymes (nanomaterials with enzyme mimetic properties) have been recently introduced as a potential diagnostic and therapeutic tool in the biomedical field. In this poster, the development of antioxidant nanozymes with an inhibitory effect on platelet aggregation as a potential therapeutic approach to treat thrombosis related diseases will be discussed.

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Gold(I)-NHC complexes: Targeting thioredoxin reductase for innovative anticancer therapy

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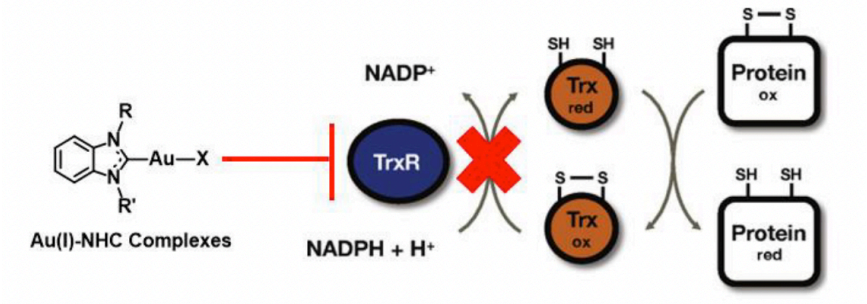
The primary aim of this study is to develop novel metal complexes as thioredoxin reductase (TrxR) inhibitors with strong anticancer potential. TrxR is a homodimeric enzyme with an N-terminal catalytic site (Cys59/Cys64) and a C-terminal catalytic site (Cys497/Sec498). It plays a vital role in maintaining cellular redox homeostasis by reducing oxidized thioredoxin to its reduced form using electrons from NADPH. Overexpression of TrxR in cancer cells is a key driver of tumor growth, as it enables these cells to manage elevated oxidative stress resulting from their rapid proliferation and high metabolic activity. Thus, targeting TrxR presents a promising therapeutic strategy to disrupt redox regulation and inhibit tumor progression.

In this study, we synthesized and characterized a series of novel Au(I)-N-heterocyclic carbene (Au(I)-NHC) complexes and evaluated their cytotoxic effects on TrxR-overexpressing cancer cell lines. Several of these complexes demonstrated potent anticancer activity, displaying low IC50 values, indicative of their efficacy in inducing cancer cell death. This study highlights the potential of Au(I)-NHC complexes as TrxR inhibitors for cancer therapy. Future research will focus on identifying the specific cell death pathways involved and further exploring the molecular mechanisms underlying their anticancer activity, with the goal of optimizing these compounds for clinical applications.



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Selenium mitigates Caerulein and LPS-induced severe acute pancreatitis by Inhibiting MAPK, NF-kB and STAT3 signalling via the Nrf2/HO-1 pathway

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Acute pancreatitis, which is inflammation of the pancreas, varies in severity and can lead to severe pancreatitis. Severe acute pancreatitis (SAP) is fatal and leads to systemic inflammation, causing multi-organ damage. Pulmonary injury, the most pertinent manifestation of the extra-abdominal organ dysfunction in pancreatitis, causes acute lung injury and acute respiratory distress syndrome, which develop in about one-third of patients; the death rate is 60%, indicating the high mortality rate due to secondary complications. Selenium is a popular antioxidant and anti-inflammatory micronutrient. Patients with pancreatitis often have selenium deficiency, and selenium supplements may provide beneficial effects. However, the role of selenium in SAP is not known. In this study, the molecular mechanisms underlying the protective effect of selenium in a mouse model of caerulein+lipopolysaccharide (cae+LPS)-induced pancreatitis was investigated. Pretreatment with selenium before the cae+LPS challenge had ameliorating effects on the SAP induced by cae+LPS. Selenium inhibited the downstream inflammatory pathways such as the MAPKs, NF-kB, and STAT3. Additionally, selenium alleviated the inflammatory condition via Nrf2/HO-1 pathway, with higher phosphorylation levels of Nrf2 in selenium-treated group. Collectively, selenium mediates anti-inflammatory and antioxidant properties by inhibiting the major inflammatory signalling pathways and activating the phosphorylation of Nrf2 pathways. These findings suggest that selenium may be a potential therapeutic option for treating SAP-associated secondary complications.

Development & evolution of essential metal (Cu, Zn & Mn) complexes of curcumin as anticancer & antioxidant agents

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The present study reports the isolation of curcumin from the rhizome of *Curcuma longa* using acetone as a solvent at temperatures between 35-40°C. Following isolation, the acetone was removed via distillation, and the residue was treated with ethanol, stirred, and filtered to obtain curcumin. Subsequently, we synthesized metal complexes of curcumin with copper, zinc, and manganese by reacting their respective metal acetates in ethanol at temperatures ranging from 80-85°C. The resulting complexes were characterized using UV-Visible spectroscopy, revealing absorption peaks at 419.5 nm for curcumin, 468.5 nm for the copper-curcumin complex, and 444 nm for the copper-curcumin methanol washings. The zinc-curcumin complex and zinc-curcumin methanol washings both exhibited an absorption peak at 444.5 nm, while the manganese-curcumin complex showed a peak at 445 nm. We also performed DPPH radical scavenging studies and assessed the cytotoxic activity of these curcumin metal complexes using the MTT assay to demonstrate their efficacy. Our findings suggest that these curcumin-based metal complexes have potential as economically viable drug candidates, warranting further exploration of curcumin's therapeutic applications.

Impact of higher dietary selenium on mineral metabolism and antioxidant status of heat-stressed rats

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Heat stress (HS) induces oxidative stress and results in lowered production and immunity in livestock. Selenium (Se) is an ultra-trace element crucial for maintaining antioxidant status of livestock. Accordingly, present study was conducted to evaluate the impact of high dietary Se levels in rats under HS. Seventy-two weaned rats were divided into six groups using a 3x2 factorial design (3 dietary Se levels and 2 temperature conditions). All groups were fed same basal purified diet, differing in Se level (i.e. CON-138ppb, Se1-291ppb, Se2-460ppb). The experiment lasted for 42 days, with all groups experiencing 28 days of thermoneutral (TN) conditions, followed by 18 days of HS condition to HS groups and TN condition throughout experiment to control groups. A metabolism trail was conducted and rats were sacrificed at the end of experimental duration. The metabolism of macro-minerals, calcium and phosphorus and trace minerals, such as zinc, copper, and manganese were unaffected ($P>0.05$) by either higher Se level or temperature conditions. As expected, the Se absorption and retention was increased ($P<0.001$) in higher dietary Se groups irrespective of temperature condition. The serum Cu and Zn levels were elevated ($P<0.05$) in rats fed higher dietary Se level under TN condition with no effect ($P>0.05$) in HS rats. Furthermore, serum Se levels were higher in rats fed high dietary Se under both HS and TN conditions. The oxidative stress was markedly enhanced in rats under HS condition, that was ameliorated by high dietary Se level. This was further supported by the increased activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase in rats fed higher dietary Se. Therefore, it can be concluded that higher dietary Se is crucial during HS condition for maintaining antioxidant status. Additionally, higher dietary Se does not have adverse effects on mineral metabolism either during TN or HS condition.

Correction of zinc deficiency in sheep by feeding zinc biofortified maize stover

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Correction of micronutrient deficiencies in soil will help in improving the health of crops as well as livestock. A field study was taken up in Zinc deficient soils in Durga Nagenhalli village of Tumkur district, Karnataka with twin objectives of studying the effect of correction of Zinc deficiency in soils on yield as well as quality of fodder maize crop and evaluating the impact of resulting zinc fortified maize fodder on improving the zinc status of sheep. Zinc sulphate was applied @ 25 kg/ha as basal dressing to the zinc deficient soil with available zinc content of 0.4 ppm and forage maize crop was grown and compared with the control field (no zinc application). The mean zinc content in fodder maize improved considerably to 36.9 ppm as compared to 26.6 ppm in control. The stover yield of maize in zinc applied plot was 26.3 t/ha compared 22.4 t/ha in control. Two groups of 10 growing sheep, deficient in zinc were fed maize stover without and with zinc fortification. The fodder and concentrate ratio was 80:20 of the total dry matter requirement in stall fed conditions for 180 days duration. The average serum zinc content of sheep significantly improved by 0.4 ppm due to feeding of zinc fortified stover. The growth rate (average daily gain) was 83 grams in fortified group sheep against 70 grams in control sheep. Similarly, after 180 days of study, the humoral immunity in fortified group of sheep against PPR vaccine was 92.6 (antibody titre, % inhibition) as compared to 85.5 in unfortified sheep. Correction of zinc deficiency in soil resulted in improving zinc content in maize fodder by over 10 ppm and feeding the resulting fortified maize fodder for six months enhanced the amount of serum zinc from 0.7 to 1.1 ppm in sheep.

Selenoproteins contribute to peripheral follicular B cell homeostasis by modulating cellular ROS levels

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Selenium, which exerts its redox regulatory effects via selenoproteins, is required for optimal functioning of the immune system. However, the role of selenoproteins in B cell homeostasis remains unclear. Here, we show that B cell selenoprotein deficiency results in reduced splenic B cell subsets and circulating serum immunoglobulins. Moreover, selenoprotein deficient B cells exhibit reduced germinal center formation and pathogen-specific antibody responses following vaccination, resulting in increased bacterial burden following challenge. Defects observed in selenoprotein deficient B cells appear to be due to an inability to control reactive oxygen species (ROS) resulting in increased cell death following stimulation. This suggests a role for selenoproteins in maintaining peripheral B cell homeostasis by regulating ROS. Interestingly, N-acetyl cysteine treatment rescued follicular but not marginal zone B cell defects indicating differential requirement for selenoproteins among these B cells. These data suggest that targeting selenoproteins may be a strategy for improved humoral immune responses.

Application of mass spectrometry-based proteomics in the analysis of trace element modified proteins: A case of selenoproteins

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To explore the potential of mass-spectrometry based proteomics workflows for the analysis of trace element modified proteins especially selenoproteins. A label-free quantification method was employed to calculate the differential proteome changes by using LC-MS/MS and a targeted proteomics methodology was tested to quantify selenocysteine (Sec) containing peptides. In this study we show that non-sec containing peptides of selenoproteins were quantified and Sec containing peptides were not detected and their ionization efficiency was low. Proteomics workflows are powerful tools to detect, identify, characterize, and quantify sec-containing peptides of selenoproteins. Nevertheless, evolving techniques and tools need to be adopted and optimized to quantify the Sec-containing peptides.

Organic and nano trace minerals at reduced dietary levels does not affect the production performance of laying hens - I

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An experiment was conducted to assess the effect of organic and nano trace minerals at reduced dietary levels on production performance of laying hens. A total of 240 Hy-Line W80 commercial layers of 29 weeks age were randomly assigned to five treatment groups with four replicates having twelve hens each. The basal diet was supplemented with inorganic trace minerals (Cu, Zn, Mn and Fe) at 100 % of requirement to form control diet or with organic trace minerals at 75 and 50 % of requirement or with nano trace minerals at 50 and 25 % of requirement to form four test diets. Each diet was offered to four replicate groups for 84 days consisting of three phases of 28 days each. Body weight and body weight changes, feed intake, total number of eggs, hen day egg production, feed conversion ratio and survivability of birds were not affected ($P>0.05$) by different dietary treatments. Egg quality parameters viz., egg weight, egg shape index, albumen index, yolk index, yolk color score, Haugh unit score, egg shell weight, albumen weight and yolk weight among the treatment groups were not affected ($P>0.05$) by source and level of trace minerals. However, egg shell thickness was significantly ($P<0.05$) higher in 25% nano trace mineral group on 28th day. It was concluded that inorganic trace minerals (Cu, Zn, Mn and Fe) in laying hen diets can be replaced with organic or nano trace minerals at 50 or 25 % inclusion level, respectively without affecting egg production and its quality with better egg egg shell thickness.

Organic and nano trace minerals at lower dietary levels improves mineral retention in laying hens

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A metabolism trial was conducted to assess the influence of dietary organic and nano trace minerals at lower levels on nutrient metabolizability and trace mineral balance in laying hens. The dietary treatments included a basal diet supplemented with inorganic trace minerals (copper, zinc, manganese and iron) at recommended levels (ITM 100), a basal diet supplemented with organic trace minerals at 75 % (OTM 75) and 50 % (OTM 50) recommend levels and a basal diet supplemented nano trace minerals at 50 % (NTM 50) and 25 % (NTM 25) recommend level. Each diet was offered to four replicates having 12 Hy-line W80 laying hens each from 29 to 42 weeks of age. A conventional total collection method employing all the birds to measure dry matter intake and excreta voided was conducted for a duration of 3 days during terminal week. The dry matter, organic matter, crude protein, ether extract and crude fiber metabolizability were similar among different diets. However, nitrogen free extract metabolizability was higher ($P<0.05$) in OTM 50 diet. The trace mineral (Cu, Zn and Mn) content in the excreta was lower ($P<0.01$) in OTM 50, NTM 50 and NTM 25 groups followed by OTM 75 group compared to ITM 100 group. However, the trace minerals (Zn and Fe) in the egg yolk were lowered ($P<0.01$) at reduced trace mineral levels (OTM 50 and NTM 25) when compared to 100 % inorganic (ITM 100) and 75 % organic trace minerals (OTM 75). The retention of minerals (Cu, Zn and Mn, except Fe) in terms of percent intake was higher ($P<0.05$) in OTM 50 and NTM 25 groups. It was concluded that inorganic trace minerals (Cu, Zn, Mn and Fe) in laying hen diets can be replaced with organic or nano trace minerals at 50 or 25 % of recommended level, respectively with better mineral retention in the body and reduced excretion and accumulation of trace minerals in the environment.

Effect of organic trace minerals at different dietary levels on nutrient digestibility and trace mineral balance in pigs

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A digestion trial was conducted to assess the effect of organic trace minerals (TM) (proteinates of Cu, Zn, Mn, Fe and Se) at different dietary inclusion levels on nutrient digestibility and trace mineral balance in pigs. The dietary treatments consisted of a basal diet supplemented with inorganic TM at 100% recommended levels (ITM 100) or with organic TM at 100 % (OTM 100), 75 % (OTM 75) and 50 % (OTM 50) recommended levels or with both inorganic TM at 50 % and organic TM 50 % recommended level (ITM 50 + OTM 50). The organic TM employed as proteinate in nature while inorganic TM used were sulphate salts of Cu, Zn, Mn and Fe and sodium selenite. 15 large white Yorkshire pigs aged 5 months were distributed into five groups of three pigs each. Each animal was housed in an independent pen and assigned with one of the five diets in completely randomized design. The duration of the trial included adjustment period for 5 days followed by collection period for 3 days. The dry matter intake and excreta voided was measured during collection period and the samples collected were analysed for proximate principles (AOAC, 2015) and trace minerals (Atomic absorption spectrophotometer). The results revealed that dry matter, organic matter, crude protein, ether extract, crude fiber and nitrogen free extract digestibility were similar among different diets. The daily retention of Cu, Zn and Mn in terms g, g/kg metabolic body weight and % of mineral intake were lower ($P<0.01$ for Cu and Zn; $P<0.05$ for Mn) in OTM 75 and OTM 50 groups. However, the retention of Fe was similar among different groups. It was concluded that inorganic trace minerals in pig diets can be replaced with organic trace minerals at lower inclusion level i.e., up to 50 % of recommended level with better retention of Cu, Zn and manganese and without affecting the nutrient digestibility.

Organic and nano trace minerals at reduced dietary levels does not affect the growth performance and nutrient metabolizability in broiler chicken

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A study was conducted to evaluate the effect of dietary supplementation of organic and nano trace minerals at reduced levels on the growth performance and nutrient metabolizability in broiler chickens. 240 day-old commercial broiler (Cobb strain) chicks were assigned in equal numbers to five groups consisting of four replicates each. The basal diet (T1) was supplemented with inorganic trace minerals at 100% of the standard recommendation to serve as control (ITM 100). Test diets were prepared by supplementing organic trace minerals at 75 (OTM 75) and 50 % (OTM 50) of the standard recommendation or nano trace minerals at 50 (NTM 50) and 25% (NTM 25) of the standard recommendation. Each diet prepared for pre-starter (1-7 days), starter (8-21 days) and finisher (22-42 days) phases was offered to respective groups. The results revealed that significantly different ($P<0.05$) body weights of the birds were observed at the end of 1st, 2nd and 3rd week while no such difference were observed at the end of 4th, 5th and 6th week. There was no significant difference ($P>0.05$) in weekly cumulative feed intake and feed conversion ratio throughout the experimental period. A metabolism trial during last week of the trial revealed that the metabolizability of proximate nutrients in broiler diets supplemented with organic and nano trace minerals at reduced levels were comparable with that of control diet (ITM 100). It was concluded that similar growth performance and nutrient metabolizability can be achieved by replacing inorganic trace minerals (Mn, Cu and Zn) with organic and nanoparticles trace minerals at 50 % and 25 % of the standard recommendation, respectively in broiler diets.

Organic and nano trace minerals at lower dietary levels improves mineral retention in laying hens

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A metabolism trial was conducted to assess the influence of dietary organic and nano trace minerals at lower levels on nutrient metabolizability and trace mineral balance in laying hens. The dietary treatments included a basal diet supplemented with inorganic trace minerals (copper, zinc, manganese and iron) at recommended levels (ITM 100), a basal diet supplemented with organic trace minerals at 75 % (OTM 75) and 50 % (OTM 50) recommend levels and a basal diet supplemented nano trace minerals at 50 % (NTM 50) and 25 % (NTM 25) recommend level. Each diet was offered to four replicates having 12 Hy-line W80 laying hens each from 29 to 42 weeks of age. A conventional total collection method employing all the birds to measure dry matter intake and excreta voided was conducted for a duration of 3 days during terminal week. The dry matter, organic matter, crude protein, ether extract and crude fiber metabolizability were similar among different diets. However, nitrogen free extract metabolizability was higher ($P<0.05$) in OTM 50 diet. The trace mineral (Cu, Zn and Mn) content in the excreta was lower ($P<0.01$) in OTM 50, NTM 50 and NTM 25 groups followed by OTM 75 group compared to ITM 100 group. However, the trace minerals (Zn and Fe) in the egg yolk were lowered ($P<0.01$) at reduced trace mineral levels (OTM 50 and NTM 25) when compared to 100 % inorganic (ITM 100) and 75 % organic trace minerals (OTM 75). The retention of minerals (Cu, Zn and Mn, except Fe) in terms of percent intake was higher ($P<0.05$) in OTM 50 and NTM 25 groups. It was concluded that inorganic trace minerals (Cu, Zn, Mn and Fe) in laying hen diets can be replaced with organic or nano trace minerals at 50 or 25 % of recommended level, respectively with better mineral retention in the body and reduced excretion and accumulation of trace minerals in the environment.