Protective Effects of Alpha Lipoic Acid (ALA) Are Mediated by Hormetic Mechanisms

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ABSTRACT

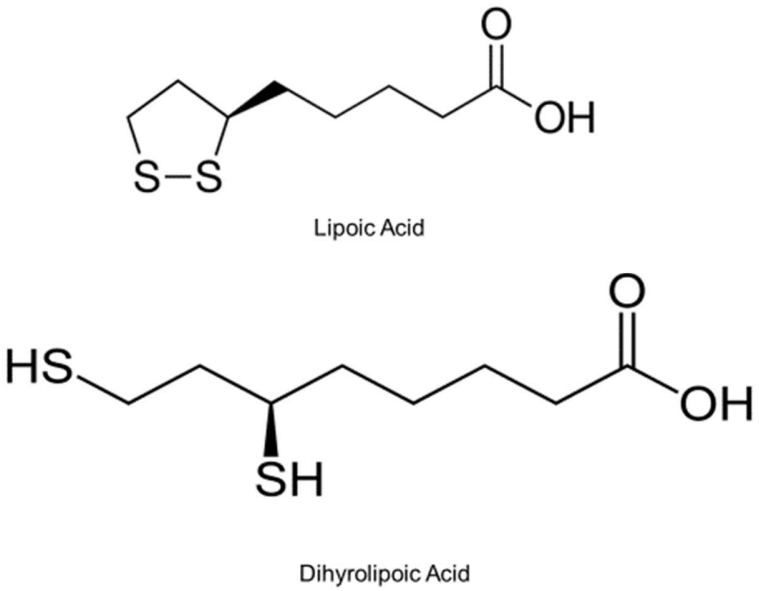
The endogenous and dietary agent, alpha lipoic acid (ALA) is evaluated for its capacity to induce a broad spectrum of adaptive responses via hormetic dose responses and their underlying mechanisms. ALA was shown to induce hormetic effects in a wide range of experimental models within *in vitro* and *in vivo* experimental settings which included direct exposure and pre- and post-conditioning experimental protocols. The hormetic effects occur in a broad range of organ systems, including the brain, heart, kidney and other tissues, with possible public health and clinical/therapeutic applications linked to reducing the onset and progression of neurogenerative diseases and also in the preservation of sperm health and functionality during cryopreservation. This paper provides the first integrated assessment of ALA-induced hormetic dose responses. Underlying mechanisms that mediated the occurrence of ALA-induced hormetic effects involved the induction of low levels of ROS that activate key cell signaling antioxidant (e.g. Nrf2) pathways.

Keywords: hormesis; alpha lipoic acid; biphasic dose response; U-shaped dose response; dietary supplements; sperm preservation

1. INTRODUCTION

Alpha lipoic acid (ALA) (1,2-dithidane-3-pentanoic acid) is synthesized in mitochondria where it serves as a co-factor for alpha keto acid dehydrogenase. ALA is an 8-carbon sulfur containing fatty acid. It is a dithiol compound that contains a chiral center at the C6 carbon position (Figure 1). The reduced form, dihydrolipoic acid (DHLA), is achieved by opening the disulfide ring (Salinthone et al., 2008). Besides this essential cellular role in energy metabolism (Salehi et al., 2019), ALA is also found in many human dietary items and distributed to many tissues following ingestion. ALA is typically found in muscle meat, heart, kidney and liver, with lower amounts in fruits and vegetables. In general, it is not likely that the average Western diet contains appreciable quantities of ALA (Shay et al., 2009; Salehi et al., 2019). Emerging evidence suggests that the ingested form of ALA is not used as a metabolic co-factor but affects a broad range of biochemical processes that have biomedical significance, acting as an antioxidant and effectively mediating and slowing various aging processes (Shay et al., 2009). As a result, ALA has been widely used as a dietary supplement, becoming the object of considerable study. While there have been excellent reviews on the effects, mechanisms and therapeutic potential of ALA (Salinthone et al., 2008; Shay et al., 2009; Salehi et al., 2019), the present assessment is designed to extend these efforts, being the first paper that explores, documents and assesses the capacity of ALA to induce hormetic dose responses and their underlying mechanisms.

Figure 1. Chemical Structure of Lipoic Acid and Dihydrolipoic Acid (Source: https://upload.wikimedia.org/wikipedia/commons/9/9f/Lipoic_acid.svg; Yikrazuul, Public domain, via Wikimedia Commons; Public Domain, https://commons.wikimedia.org/w/index.php?curid=1397101



2. HORMETIC DOSE RESPONSE OVERVIEW

While hormesis has been substantially documented and evaluated in the biological, toxicological and biomedical literature, the use of the term hormesis (or hormetic responses) in the ALA literature is very limited despite the widespread presence of hormetic-like biphasic dose responses. This restricts standard key word search methods, and further suggests the need to provide an overview of the hormesis concept in order to clarify better the hormesis concept and its relevance to ALA in the present paper.

Hormesis is a biphasic dose/concentration response, in which low-doses/concentrations induce stimulation, and high-doses/concentrations cause inhibition (Calabrese and Baldwin, 2002; Calabrese and Mattson, 2011; Calabrese, 2008; Mattson, 2008). The quantitative characteristics of hormesis are characterized by a maximum stimulatory response, usually 30% to 60% greater than the concurrent control comparison group (Figure 2), with a stimulatory width that is typically in the 10-20 fold range (Calabrese et al., 2019). However, the stimulatory width may show substantial variability, and it is not unusual for it to exceed 50 fold. The hormetic response may be induced by a direct subtoxic (hormetic) dose, and/or subtoxic (hormetic) preconditioning dose, which is followed by a toxic dose (Calabrese 2016a,b), or an overcompensation to a disruption in homeostasis (Calabrese, 1999, 2008). Hormesis displays substantial generality, and is independent of biological model, inducing agent, endpoint and mechanism (Calabrese, 2013) with a long history within the chemical and radiation biology research areas (Calabrese and Baldwin, 2000a-e). Figure 2 illustrates the hormetic dose response (Calabrese and Baldwin, 1998).

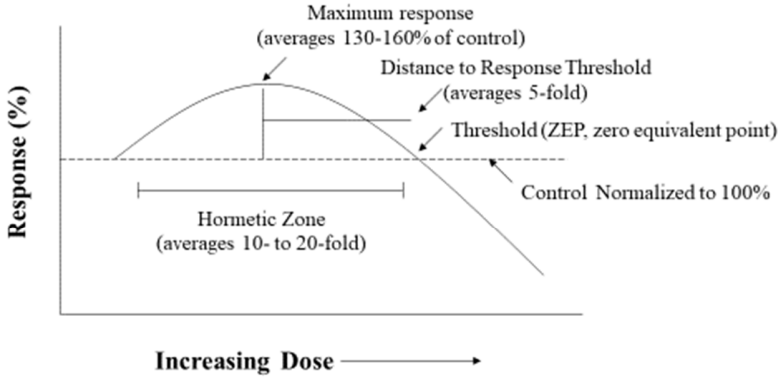


Figure 2. General representation of the hormetic dose response (modified from: Calabrese and Baldwin, 1998)

3. BIOMEDICAL TOPICS

3.1. Sperm

In 1982 Alvarez and Storey reported that the process of thawing cryopreserved sperm leads to the formation of reactive oxygen species (ROS). The production of excessive ROS in this manner decreases the quality of the thawed spermatozoa (Stradaioli et al., 2007). Since the production of excessive ROS during this cryopreservation thawing process appears to exceed the capacity of the natural antioxidant system to fully protect such thawed sperm, synthetic antioxidant agents have been explored to reduce the toxicity of the ROS stress and thereby enhance the quality of the cryopreserved sperm (Asadpour et al., 2012). The use of well-known synthetic antioxidants such as BHT protect the sperm of turkeys (Donoghue and Donoghue, 1997), goats (Khalifa et al., 2008), dogs (Sahashi et al., 2011) and bulls (Asadpour et al, 2012). Similar assessments have been also directed to other agents, such as alpha tocopherol (Ullah et al., 2019), linoleic acid (Buyukleblebici et al., 2014), glycine (Nazif et al, 2022), and coenzyme Q10 (Ibrahim et al., 2011). Since ALA in its reduced form, dihydrolipoic acid (DHLA), can prevent the harmful effects of oxidative stress in aqueous and membranous contexts as well as affecting the regeneration of vitamins C & E, Ibrahim et al. (2008) explored the use of ALA to optimize sperm function for infertile couples. This concept was later extended to the area of animal husbandry and cryopreservation with a series of papers starting in 2015 showing consistent hormetic dose responses with a broad range of animal model sperm [e.g., goat, Ren et al., 2018 (Figure 3); rooster, Najafi et al., 2021 (Figures 4A and 4B); carp, Inanan and Kanyilmaz, 2020 (Figure 5); boar, Ibrahim et al., 2008, Shen et al., 2015 (Figure 6); and bull, Ayaz et al., 2021; Ahman et al., 2018 (Figures 7 & 8A, 8B)]. An assessment of these studies indicates that they display similar methodologies but with some modest tailored modifications.

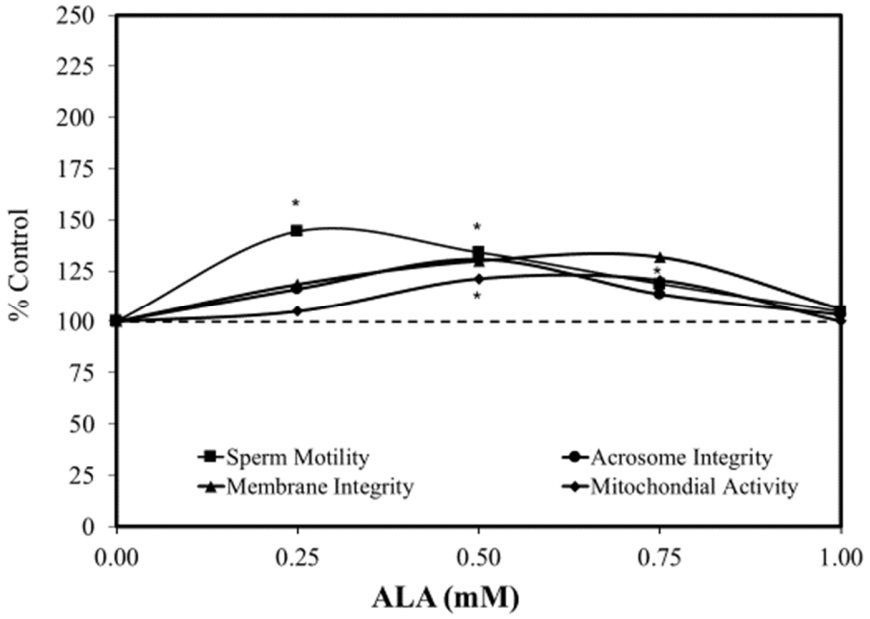


Figure 3. Effects of alpha lipoic acid (ALA) on cashmere goat cryopreservation sperm (modified from: Ren et al., 2018) *P= ≤ 0.05

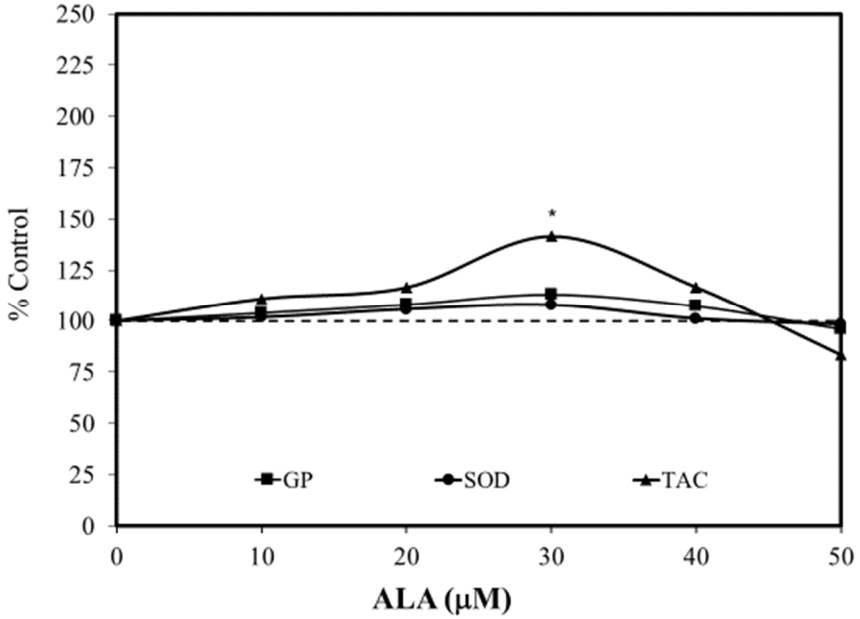


Figure 4A. Effects of alpha lipoic acid (ALA) on GP, SOD, and TAC of post-thawed rooster semen (modified from: Najafi et al., 2021) *P= ≤ 0.05

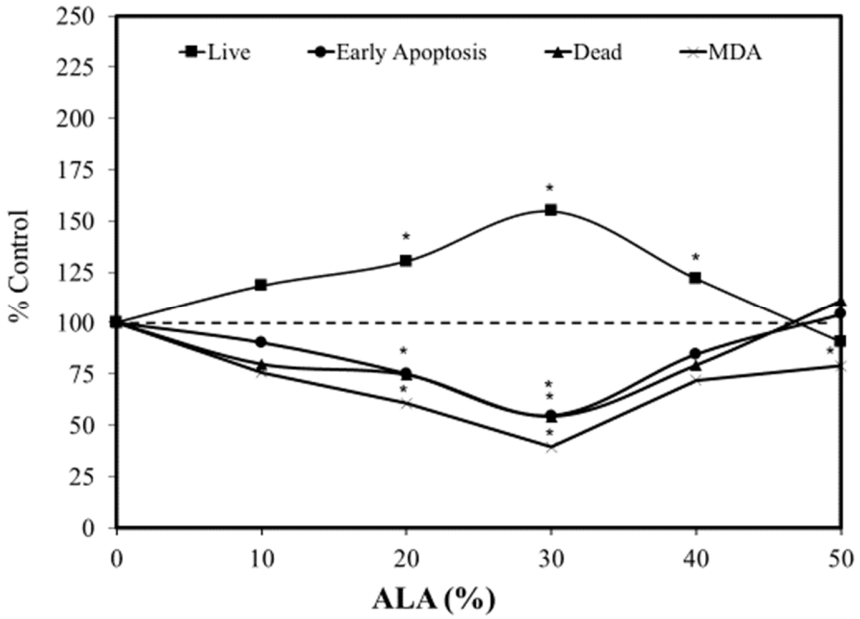


Figure 4B. Effects of alpha lipoic acid (ALA) on live, apoptosis, death spermatozoa in rooster thawed semen (modified from: Najafi et al., 2021) *P= ≤ 0.05

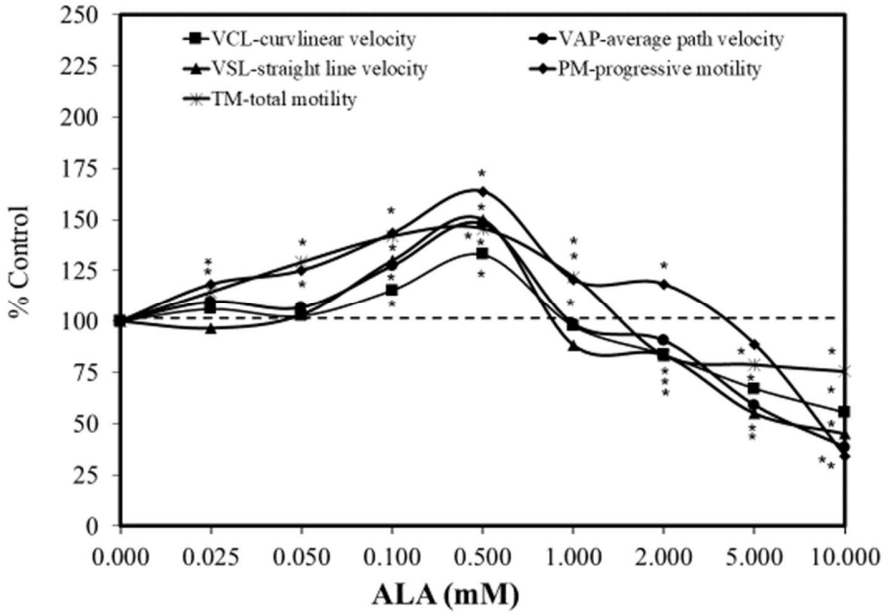


Figure 5. Effects of alpha lipoic acid (ALA) on motility parameters of common carp spermatozoa (modified from: Inanan and Kanyilmaz, 2020) *P= ≤ 0.05

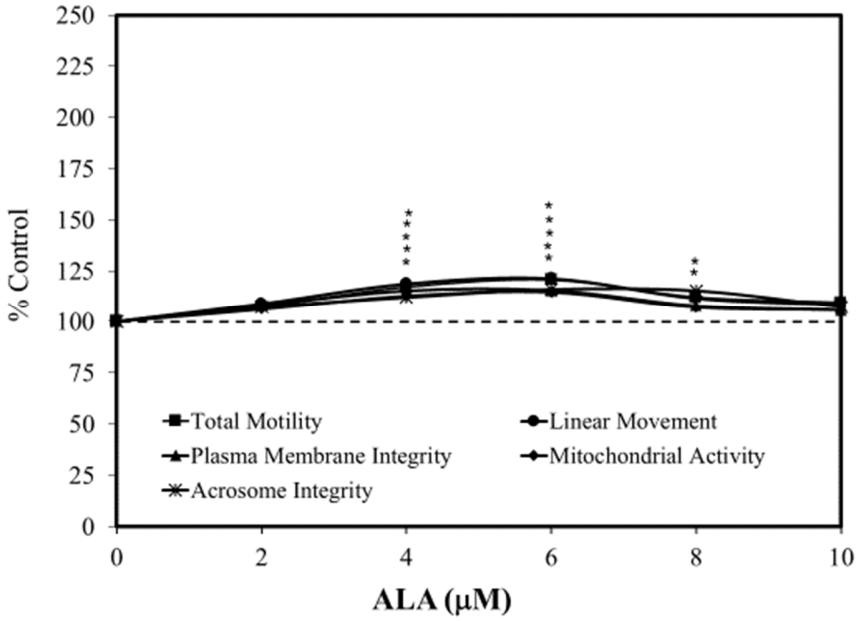


Figure 6. Effects of alpha lipoic acid (ALA) on boar sperm (modified from: Shen et al., 2015) *P= ≤ 0.05

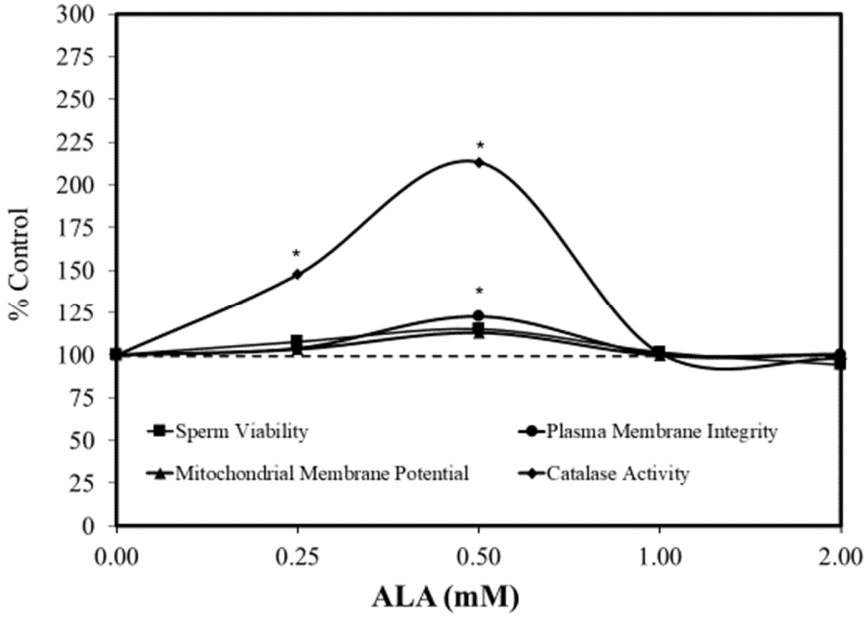


Figure 7. Effects of alpha lipoic acid (ALA) on bull spermatozoa on post-thaw biological performance and post-thaw evaluation (modified from: Ayaz et al., 2021) *P= ≤ 0.05

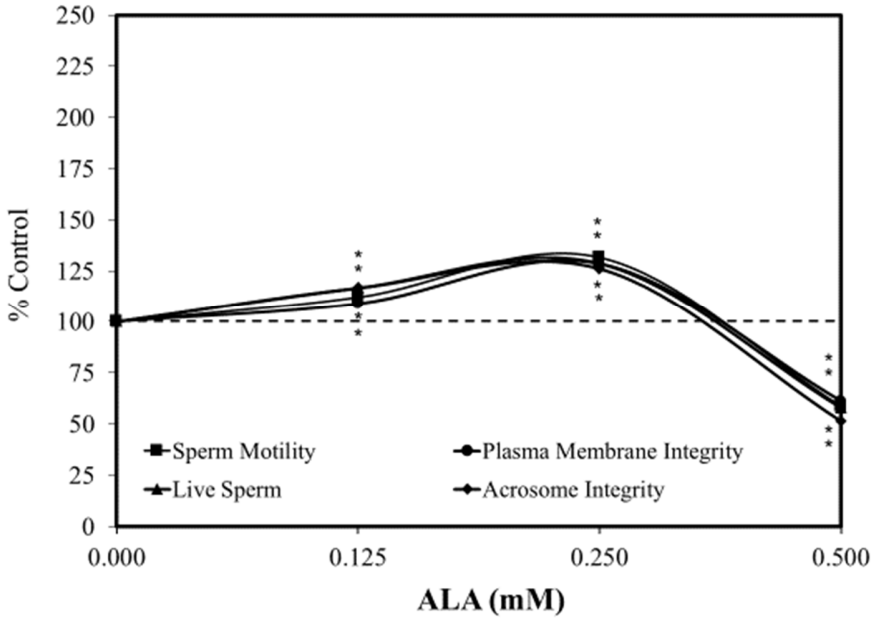


Figure 8A. Effects of alpha lipoic acid (ALA) to extender of pre-freezing value of bull spermatozoa (modified from: Ahmad et al., 2018) *P= ≤ 0.05

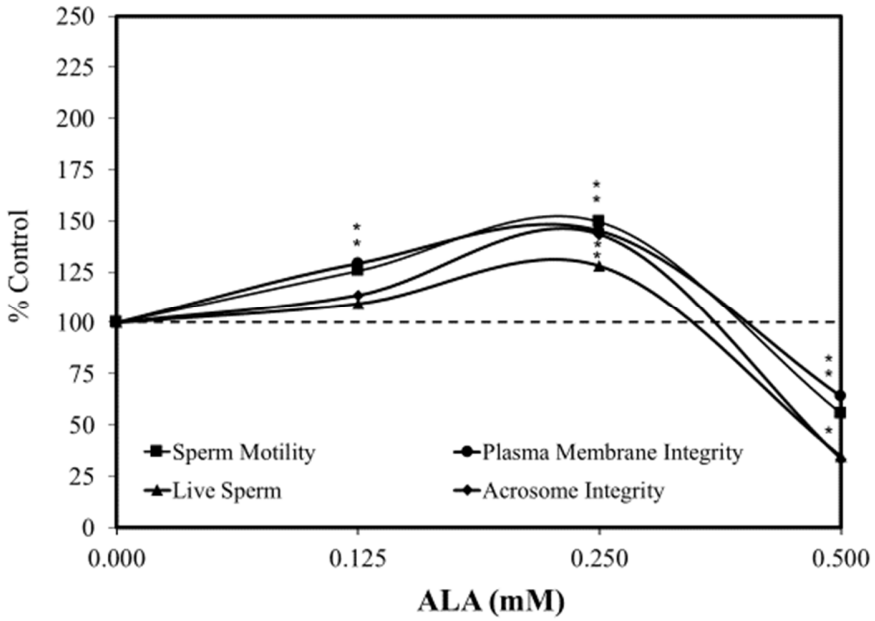


Figure 8B. Effects of alpha lipoic acid (ALA) to extender of post-thaw value of bull spermatozoa (modified from: Ahmad et al., 2018) *P= ≤ 0.05

These experiments generally used pooled sperm from all males. The sperm needed to showed a minimum degree of performance quality which, for sperm motility, ranged from greater than 65 to less than 80% depending upon the study. The analysis of sperm counts per ml was in the 1-40 x 10^6 per ml range. The ALA was dissolved in 0.1 ethanol (Inanan and Kanyilmaz, 2020) or with sodium hydroxide (Najafi et al., 2015) or was not indicated by other papers. The processes of thawing were generally similar allowing for 30 seconds at 37^0C. Other papers such as Shen et al. (2015) used 37^0C for 45 seconds while Inanan and Kanyilmaz (2020) used 25^0C and 10 seconds. The range of ALA concentrations assessed was unique to each animal model studied but was generally in the range of 0.1 to 10 mM. In addition to measuring a broad range of functional motility endpoints, most studies also assessed a range of oxidant and antioxidant biomarkers (e.g., SOD, GSHpx, catalase, and LDH). The findings indicated hormetic effects on the sperm function and antioxidant parameters, with the quantitative features across these studies being quite similar. The median stimulatory capacity was 130.7%, with the median stimulatory width being 3-fold. The ALA therefore enhanced the functional capacity of sperm in an hormetic fashion and it did so while upregulating antioxidant enzyme capacities.

4. NEUROPROTECTION

4.1. PC12 Cells

Diabetes mellitus is associated with hyperglycemia and altered secretion and/or actions of insulin. The occurrence of chronic hyperglycemia is usually linked to various microvascular dysfunctions affecting multiple organs such as the kidney, retina, as well as peripheral nerves. The mechanism of cell toxicity is related to the fact that high glucose concentrations enhance

cellular generation of ROS/NOS. This process leads to an altered balance between the production of ROS/NOS and antioxidant capacity, affecting cellular resiliency and susceptibility to cell death via apoptosis. Efforts have been made to find agents that may protect or prevent high glucose level-induced neurotoxicity. ALA has attracted some interest due to its lipophilic and hydrophilic features, and its capacity to cross cell membranes and the blood brain barrier (BBB). ALA, and its reduced form, DHLA, have a redox potential of -0.32 V which exceeds that of GSH/GSSH (-0.24 V). As a result of these unique chemical features and protective preliminary studies in various models of neuronal injury, Najafi et al., (2015) assessed the effects of ALA on PC12 cells within a preconditioning experimental protocol. In this study PC12 cells were pretreated with a range of ALA concentrations over a period from 16 to 48 hours. For these PC12 cells a high concentration of glucose (25 mg/ml) decreased cell viability by about 25%. ALA was unable to prevent the induced toxicity with the 16 hour pretreatment but did so with a 24 and 48 hour preconditioning periods. The lowest ALA concentration (100 μM) enhanced the recovery of the cells (Figure 9), with the 24-hour period being optimal. The optimal protection concentration and time prevented apoptosis and restored key antioxidant enzymes such as catalase and SOD.

4.2. Glutamate Toxicity

Astrocytes exhibit a broad spectrum of adaptive roles in the mammalian nervous system. These include a range of key functional outcomes in neurons, such as providing trophic support, antioxidant defenses, as well as facilitating the uptake of amino acids (Kleinkauf-Rocha et al., 2013). The astrocyte has a major role in glutamate transport, the regulation of extracellular glutamate levels and the passaging of glutamate to neurons in the glutamine form. The astrocyte also has an essential role in the maintenance of GSH levels, the principal antioxidant of the brain.

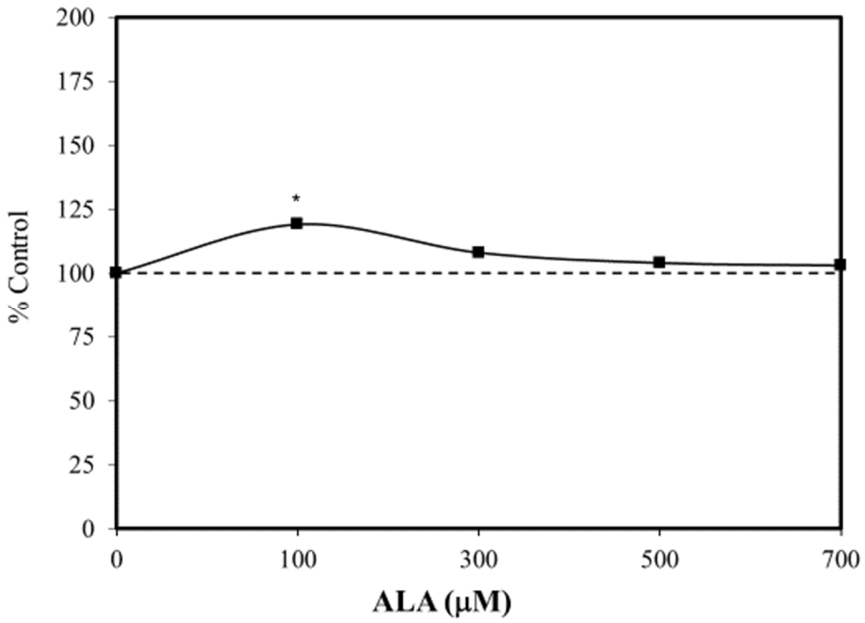


Figure 9. Effects of alpha lipoic acid (ALA) pretreatment (24 hours) on high glucose induced toxicity in PC12 cell measure by cell viability (MTT assay) (modified from: Najafi et al., 2015)
*P= $\leq$ 0.05

In light of these numerous key astrocyte roles, Kleinkauf-Rocha et al. (2013) evaluated the capacity of the ALA/DHLA system to affect various factors such as maintaining GSH concentrations as well as various glutamate functions such as amino acid uptake and enzyme activities. Of particular interest is that Kleinkauf-Rocha et al. (2013) reported that ALA-induced J-shaped concentration effects for ROS and nitrite production (Figure 10). In a similar manner the ALA enhanced the uptake of glutamate, increased GSH content and glutamine synthetase activity, each showing similar hormetic dose responses. The authors noted that ALA can significantly affect astrocyte parameters that are related to oxidant/pro oxidant effects. These findings, which represent the first report that ALA may affect glutamate uptake and glutamate synthetase, a modulator of GSH production, suggest that ALA may slow the onset of neurodegenerative diseases and enhance recovery from brain damage.

4.3. Alzheimer's Disease

Interest in whether ALA might be of value in the treatment of Alzheimer's disease (AD) first occurred following reports that it improved learning and memory in rat models (Shinto et al., 2014; Galasko et al., 2012). The enhanced learning was also associated with increased glucose metabolism and insulin sensitivity. A follow-up pilot study with nine AD patients by Hager et al., (2001) indicated that orally administered ALA for one year significantly improved cognitive function in a battery of neuropsychological tests. Consistent with the clinical findings was an *in vitro* study by Zhang et al. (2001) which found that ALA pretreatment (48 hours) prevented beta amyloid (Aβ) protein induced neuronal damage via activation of the PKB/Akt signaling pathway. Likewise, co-exposure of ALA prevented Aβ protein aggregation, reduced Aβ fiber formation and depolymerized the aggregated Aβ (Ono et al., 2006). As a follow-up to

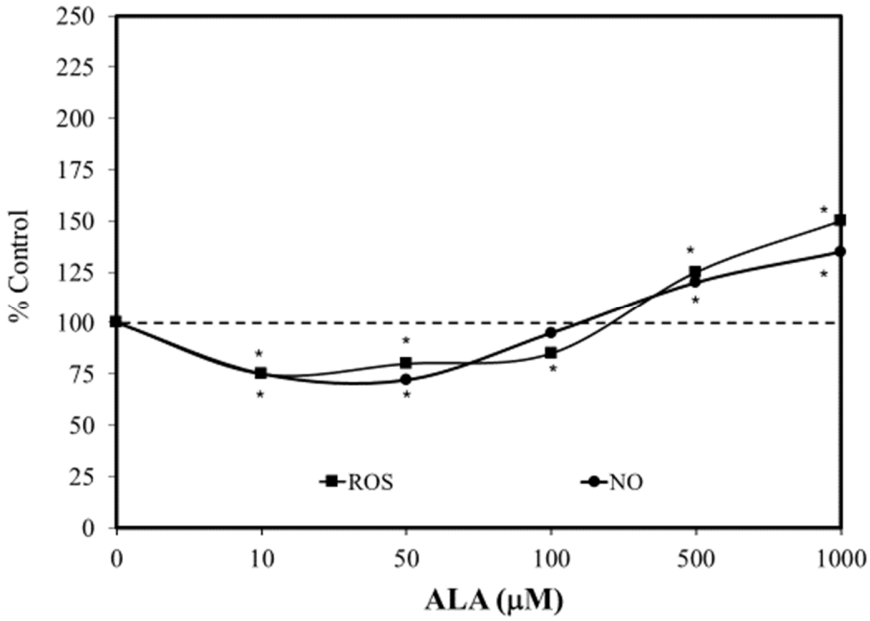


Figure 10. Effects of alpha lipoic acid (ALA) on reactive oxygen species (ROS) production and nitric oxide (NO) levels in C6 astrocytes (modified from: Kleinkauf-Rocha et al., 2013) *P= ≤ 0.05

these investigations Niu et al. (2018) reported that ALA protected SH-SY5Y cells from toxicity to 160 µg/ml AGE-BSA advanced glycation end products in a preconditioning protocol (i.e., 30 minutes preconditioning) (Figure 11). Based on the results of the MTT assay, LDH leakage and axonal length, each endpoint showed an hormetic dose response. Follow up research by Collins et al. (2023) indicated that pretreatment (24 hours) with a combination of ALA (0.2-200 µM) and noringenin (0.2-200 µM) called VANL-100 significantly protected against toxicity to SH-SY5Y cells in an hormetic manner that were treated with $A\beta_{25-35}$ (Figure 12). However, when both compounds were tested separately in a direct exposure (i.e., non-pretreatment) protocol neither had an effect on the viability of the SH-SY5Y cells. Both agents showed apparent hormetic effects when tested separately when pretreated (24 hours) prior to exposure to $A\beta_{25-35}$. The magnitude of the hormetic stimulation was modestly greater when the two compounds were combined.

4.4. Hearing Loss

Hearing loss is a serious public health and medical concern. There are numerous causes of hearing loss, including genetic predisposition, age, viral infections, and some anti-cancer and antibiotic agents. Considerable efforts have been directed to finding treatments that may minimize/prevent such induced auditory damage. These include a wide range of dietary and supplementary strategies and preconditioning approaches, using diverse chemical, physical, and physiological tactics. Several reviews have addressed many of these approaches (Pak et al., 2020; Abbasi et al., 2021; Mahmoudian-sani et al., 2019). Due to its strong antioxidant capacity, ALA has also been assessed for its capacity to prevent cisplatin-induced ototoxicity in the rat model. For example, Rybak et al. (1999) reported that ALA (100 mg/kg-IP) prevented cisplatin

(19 mg/kg-IP) induced auditory damage. In a complementary fashion, ALA prevented cisplatin induced ototoxicity via intra-tympanic application, thereby bypassing systemic influences (Ozkul

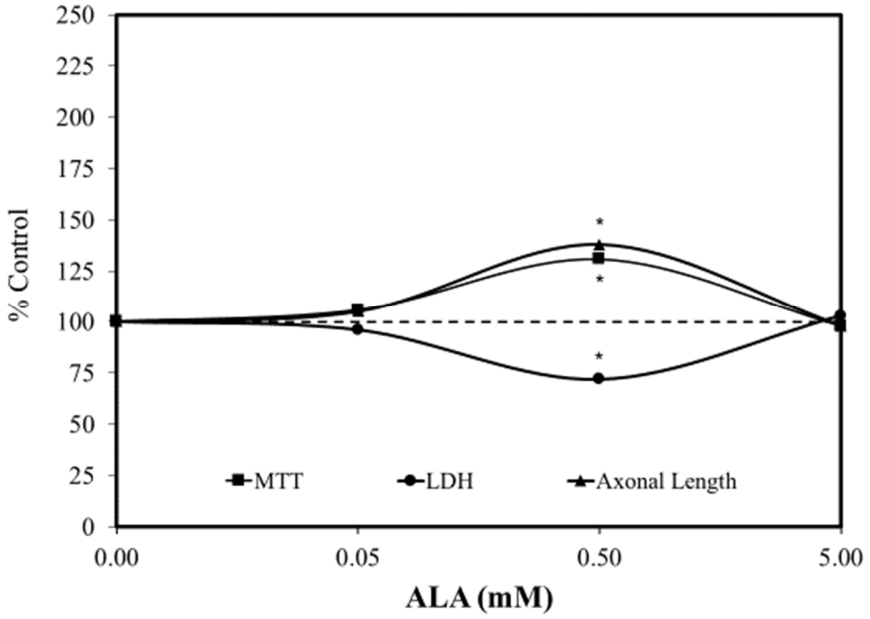


Figure 11. Effects of alpha lipoic acid (ALA) on SH-SY5Y on preconditioning protocol (modified from: Niu et al., 2018) *P= $\leq$ 0.05

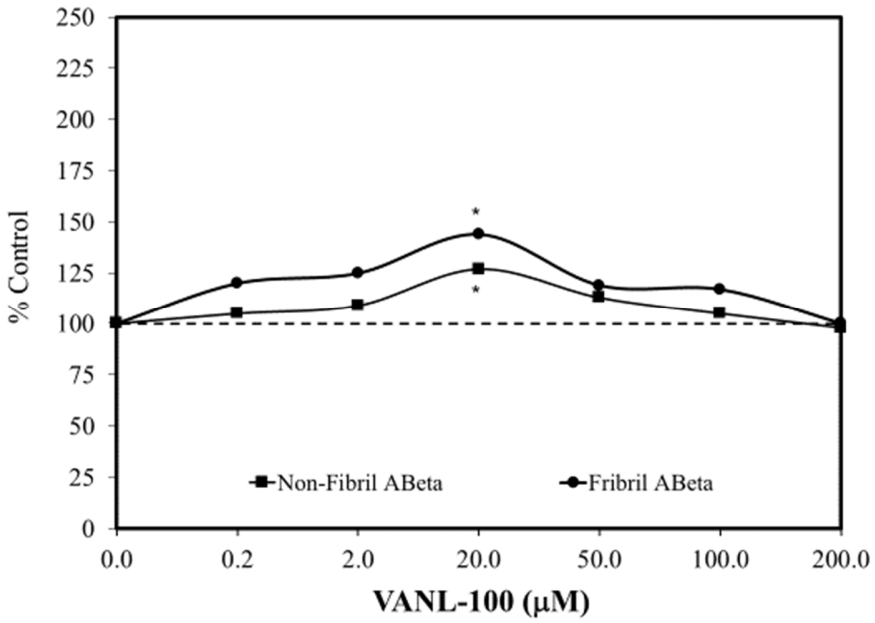


Figure 12. Effects of antioxidant compound VANL-100 (co-treatment of ALA and noringenin with each agent given at the concentration reported in the figure) on $A\beta_{25-35}$ (24 hours) in SH-SY5Y cells (modified from: Collins et al., 2023) *P= $\leq$ 0.05

et al., 2014). A subsequent report by Koo et al. (2016) indicated that ALA enhanced cell viability of HEI-OC1 cells, an auditory cell line, in an hormetic-biphasic manner (Figure 13), and also in 24-hour preconditioning. The optimal concentration range was similar in both experiments (direct and preconditioning) being in the 100-400 µM range. However, while the 500 µM concentration displayed a return to baseline value in the direct exposure experiments (Figure 13), this concentration in the preconditioning experiments was still protective (Figure 13). The ALA protective treatments reduced the capacity of cisplatin to induce ROS formation. This was the case with respect to reducing the occurrence of apoptosis related caspase activities (caspase 3, 8, 9) as well as with poly(ADP-ribose)polymerase (PARP) cleavage, which enhances cellular apoptosis. Complementary to these experimental studies was a clinical trial with ALA in which 10 days of 600 mg/day ingestion was associated with a significant level of protection at 6 kHz of Transcient Evoked Otoacoustic Emissions (TECE) (Quaranta et al., 2012).

5. CORNEAL CELLS – GLUCOSE TOXICITIES

5.1. Advanced Glycation End Products (AGEs)

Advanced glycation end products (AGEs) are irreversible adducts from glucose-protein condensation reactions, in addition to lipids and nucleic acids exposed to reducing sugars. Conditions such as hyperglycemia and/or aging are closely associated with the formation of AGEs. When AGE-molecules bind to their receptor it leads to the formation of ROS and the development of oxidative stress via activation of specific sets of transcription factors. Since this activity is central to the development of pathological processes in those with diabetes mellitus, it led to an exploration of whether ALA may block such AGEs-induced oxidative stress and related

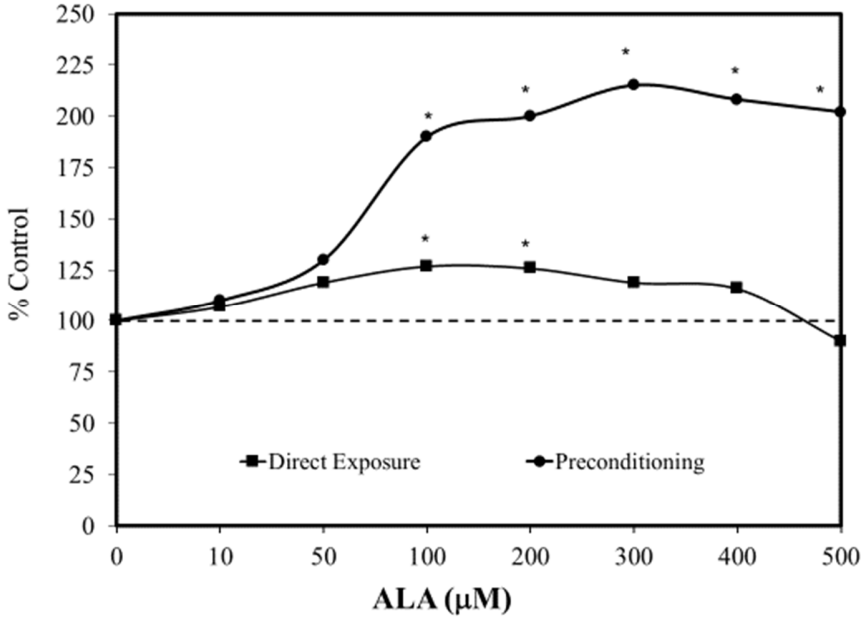


Figure 13. Effects of alpha lipoic acid (ALA) on the viability (CCK-8 assay) of HEI-OCI cells, an auditory cell line (modified from: Koo et al., 2016) *P= ≤ 0.05

inflammatory processes. Using human corneal epithelial cells as a model, Li et al. (2022a) initially tested the effects of a broad range of ALA concentrations on cell proliferation using the CCK-8 assay, which quantifies the number of cells by producing an orange formazan dye upon bioreduction in the presence of an electron carrier (Figure 14). The glucose treatment by itself displayed an hormetic biphasic concentration response with 10 mM being optimal. Using the lowest concentration of glucose tested (i.e. 5 mM-no treatment effect) within the cell culture media, ALA enhanced human corneal epithelial cell proliferation in an hormetic fashion with the optimal concentration being 50 µM. The 25 µM ALA concentration blocked the capacity of high glucose 25 mM to induce the formation of AGEs and the activation of AGE receptors. Consistent with these findings, ALA (25 µM) prevented the elevated glucose treatment from inducing oxidative stress, reducing antioxidant enzyme activity and a series of biomarkers of inflammation. While the 25 µM ALA concentration was effective in blocking the adverse effects of the high glucose treatment, it would have been of value to have tested the response over a broader concentration range. Also, the ALA concentration of 25 µM was closely related to *in vivo* treatment concentrations such as the concentration in the blood for a person ingesting 600 mg of ALA at one time (i.e., 33 µM), which is common.

5.2. Kidney - Podocytes

Diabetes mellitus is a serious and widespread disease that is characterized by the occurrence of diabetic nephropathies and neuropathies. With respect to diabetic nephropathy, alterations in podocyte (a highly specialized epithelial cell type of the visceral layer of the Bowman Capsule which affects capillary filtration) function has been associated with disease progression (Carney, 2016). The functioning of podocytes is significantly influenced by the structural proteins of various transcription factors (e.g., NF-kB and zinc finger proteins). In the

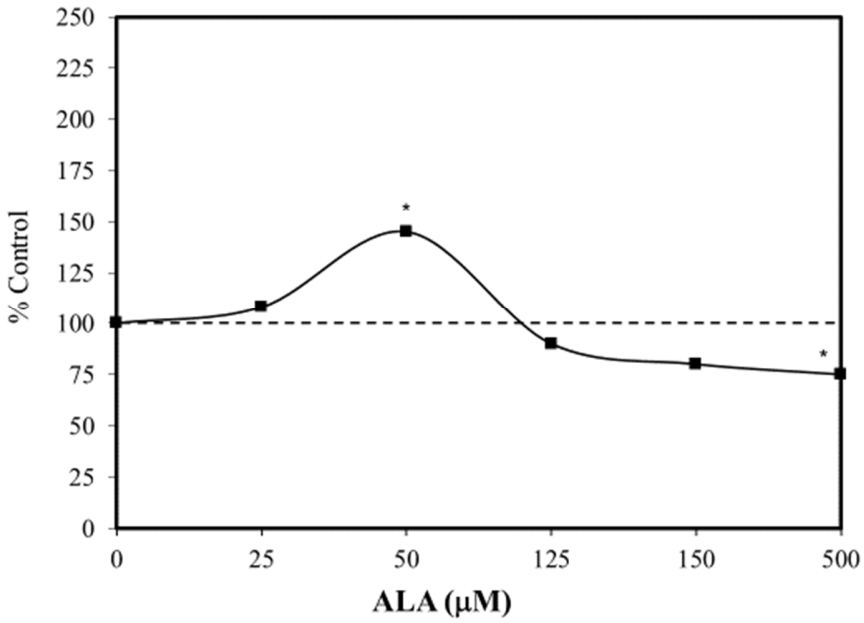


Figure 14. Effects of alpha lipoic acid (ALA) on cell proliferation in human corneal epithelial cell lines (modified from: Li et al., 2022a) *P= $\leq$ 0.05

case of the kidney protein nephrin, its expression and phosphorylation decreases in multiple renal diseases (Carney, 2016).

ALA is approved for treatment of diabetic related neuropathy (Chong and Hester, 2007), being a safe and effective treatment. In addition, ALA can also mediate the progression of diabetic nephropathy in animal models (Yi et al., 2012) and has been effective in a number of human trials, with eleven being reviewed by Sun et al. (2021) following a systematic review. The ALA doses used in these studies ranged from 300 to 600 mg/day. The mechanism by which ALA mediated its protective effects involves, at least in part, the negative regulation of NF-kβ, leading Leppert et al. (2017) to assess the effects of ALA on the viability of podocytes. Using immortalized human podocytes, they reported that ALA enhances the viability based upon the trypan blue assay in an hormetic biphasic manner (Figure 15A). Likewise, ALA also hormetically mediated the expression of zinc finger proteins at the optimal stimulatory concentration for cell viability (Figure 15B). While the effects were studied in a non-diseased model, it would be of importance to assess whether ALA could enhance the adaptive capacity of podocytes in a kidney disease model.

6. HEART

Cardiomyopathy within diabetic patients is a serious condition which develops due to prolonged hyperglycemia, involving cardiac apoptosis, with the loss of contractile units. This process involves the occurrence of compensatory hypertrophy of myocardial cells and reparative fibrosis. Yao et al. (2012), therefore assessed the capacity of ALA to prevent oxidative stress induced cardiac apoptosis using rat cardio-myoblast H9c2 cells, in the context of D-glucose

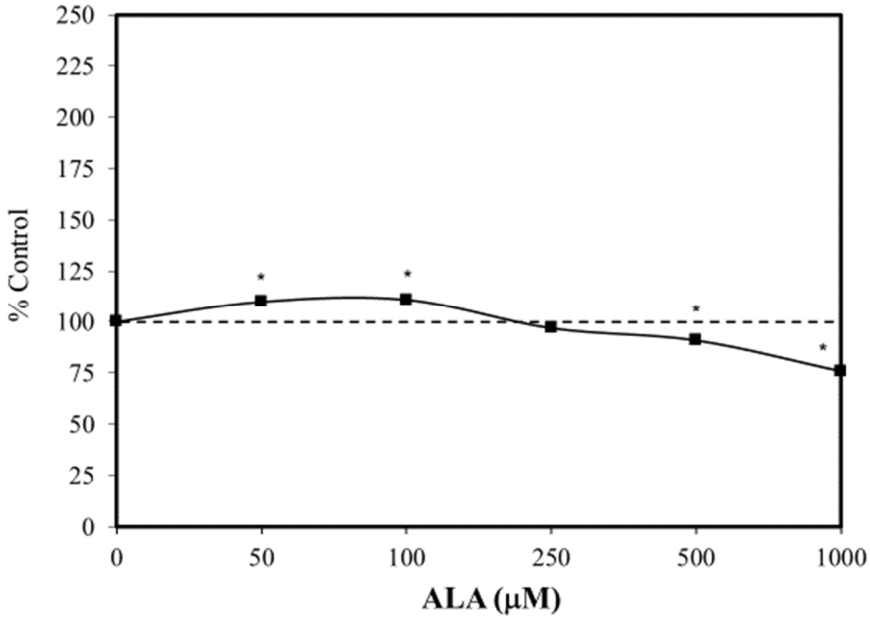


Figure 15A. Effects of alpha lipoic acid (ALA) on human podocyte (hPC) cell viability (Trypan Blue staining assay) (modified from: Leppert et al., 2017) *P= ≤ 0.05

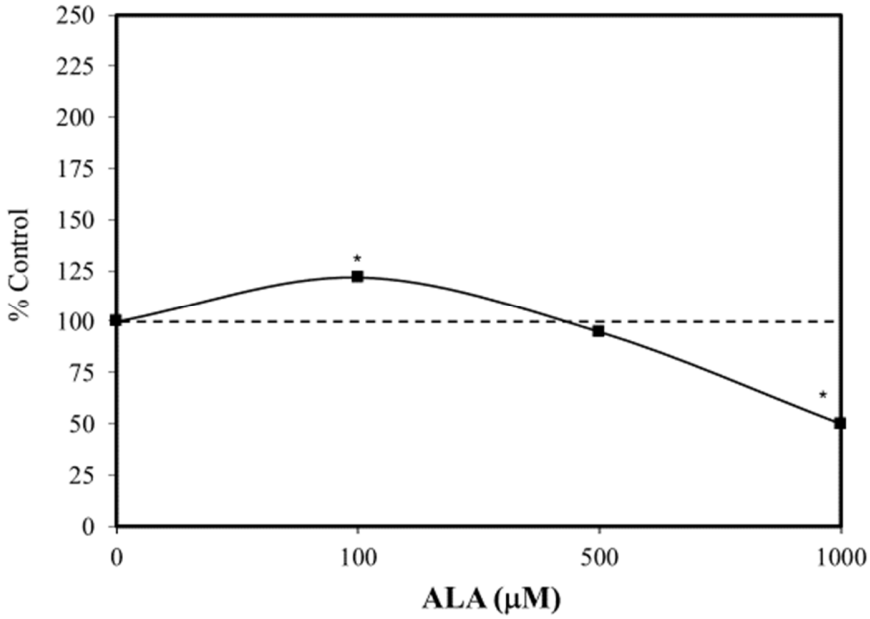


Figure 15B. Effects of alpha lipoic acid (ALA) on the expression of zinc finger protein 582 (ZNF580) (modified from: Leppert et al., 2017) *P= ≤ 0.05

(DG)/glucose oxidase (GO) induced injury. They found that ALA enhances the viability of the DG/GO treated cells in an hormetic-like biphasic concentration fashion in a direct exposure protocol (Figure 16). Note that the $\geq$ 500 µM concentration employed in this study would require a dose approximately 10-fold higher than that currently approved for human use. Further, using the optimal stimulatory concentration of the previous study they showed that a 12 hour preconditioning exposure to ALA prevented the toxicity of DG/GO to the H9c2 cells. Mechanistic experimentation revealed that ALA activated ERK1/2 while modestly enhancing ROS production. When the ERK1/2 activation by ALA was blocked by pathway inhibitors, the ALA protection was abolished. Likewise, the administration of N-acetyl cysteine (NAC) blocked the ERK1/2 induced activation, preventing the cytoprotection. Of particular interest was that the increase in ROS by ALA was at a rate that was much slower than that induced by the DG/GO treatment. The ALA induced cell proliferation and protection in the preconditioning experiment therefore depends on the formation of low concentrations of ROS to act in the hormetic fashion.

7. STEM CELLS

Whether ALA may affect the development of human pluripotent stem cells was investigated by Dong et al. (2020), who assessed the role of low levels of ROS in self renewal and maintenance of hematopoetic stem cells. Using human embryonic stem cells, the ALA was administered over a broad concentration range (50 to 800 µg/ml) to induce hematopoetic differentiation based on changes in the number of $CD34^+$ and $CD34^-$ hemogenic endothelium cells. At concentrations less than 200 µg/ml, ALA stimulated the production of endothelial cells. The responses followed an hormetic-biphasic concentration response (Figure 17) that was consistently shown at 8 and 10 days. These findings indicate that ALA may promote

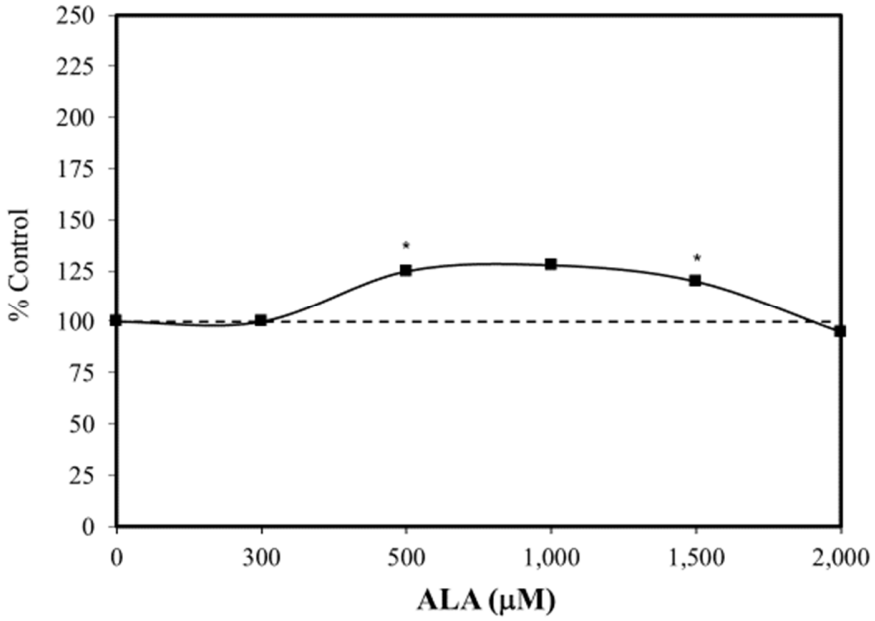


Figure 16. Effects of alpha lipoic acid (ALA) on the viability of rat cardiomyoblast D-glucose/glucose oxidase (DG-GO) treated H9c2 cells (MTT assay) (modified from: Yao et al., 2012) *P= ≤ 0.05

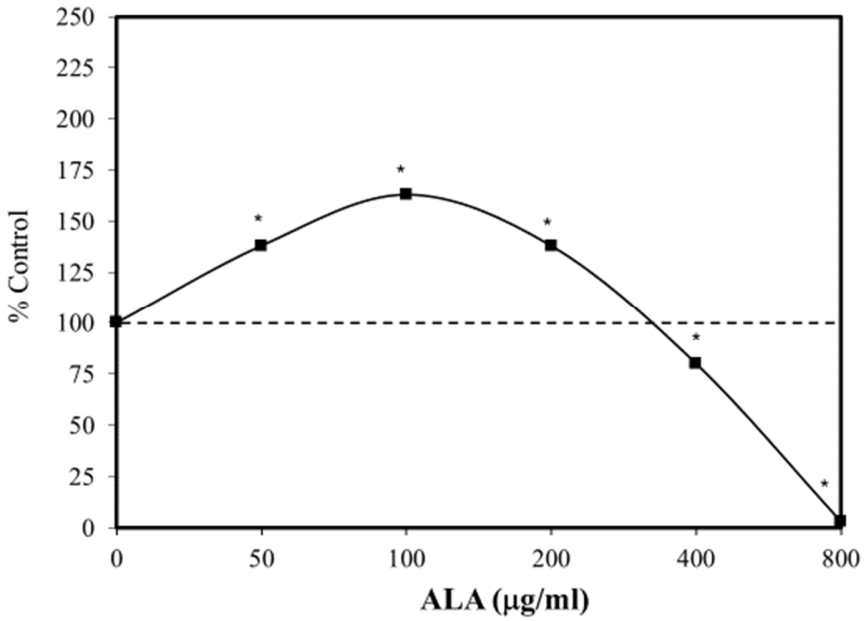


Figure 17. Effects of alpha lipoic acid (ALA) on the production of hemogenic endothelium cells (cell number counts) (modified from: Dong et al., 2020) *P= ≤ 0.05

hematopoiesis during the early stages of the endothelium to hematopoetic transition. The ALA acts via the regulation of both ROS and apoptotic related pathways.

8. SKIN - MELANIN

Melanin is synthesized in epidermal melanosomes by the actions of the amino acid tyrosine. The excessive production of melanin can become an aesthetic concern and often treated with depigmenting agents. Alpha lipoic acid has been used as a depigmenting agent due to its capacity to inhibit the expression of melanogenesis inducing tyrosinase enzymes. *In vitro* comparison of four anti-pigmentation agents, including ALA, was reported by Martinez-Gutierrez et al. (2014) using human skin melanocytes assessing cell viability. Of particular interest is that each of the four agents enhanced cell viability showing hormetic biphasic dose responses (see Figure 18 for ALA effects). However, each showed a unique optimal stimulatory concentration range. Of interest was that the ALA was tested in paired combination with the three other agents. Despite these three sets of interaction experiments only the ALA and kojic acid experiment evaluated the optimal stimulatory concentrations of both agents together. This combination resulted in a decreased stimulatory response compared to either agent acting alone. The effect of ALA and the other three agents were tested only at the highest dose that did not suppress tyrosinase activity. In these four instances only the agent arbitan was tested at a dose that was also hormetic. The dose that affected a 45% increase in cell viability was associated with a 25% decrease in tyrosinase activity.

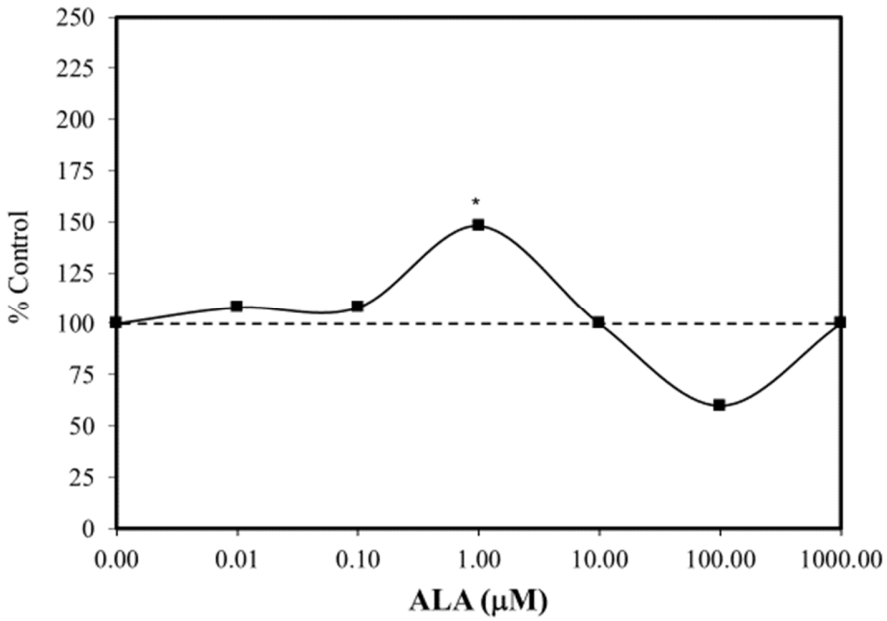


Figure 18. Effects of alpha lipoic acid (ALA) on the viability of human skin melanocytes (modified from: Martinez-Gutierrez et al., 2014) *P= ≤ 0.05

9. TOXIC SUBSTANCES

9.1. Arsenic

Arsenic-induced toxicity typically targets the mitochondria, altering the functions of the respiratory chain, generating enhanced ROS production, reduced mitochondrial membrane potential and increased membrane lipid peroxidation. Since ALA is a powerful antioxidant, Mozaffarian et al. (2022) evaluated whether it would blunt the toxicity of arsenic using mitochondria isolated from the liver of male Wistar rats (230-260 gm). In the absence of arsenic, the ALA enhanced the viability of isolated mitochondria after 15, 30 and 45 minutes, showing hormetic dose responses (Figure 19). Follow up experiments using the optimal concentration of ALA at each time period within a preconditioning protocol significantly prevented the arsenic induced mitochondrial toxicity.

9.2. Lead

Alpha lipoic acid is a well-known neuroprotection agent. It has enhanced cognition and reversed oxidative stress in 12 month old SAM P8 mice (Farr et al., 2003) and restored the induction and maintenance of long term potentiation (LTP) in aged mice (McGahon et al., 1999). Such observations led Wang et al. (2008) to assess the capacity of ALA to protect against lead induced impairment of LTP in rats. Using male and female young adults as models, the ALA treatment fully reversed the capacity of lead to suppress LTP. These changes were associated with increases in antioxidant potential, such as SOD and GSH, displaying hormetic dose responses in each case (Figure 20). These findings were particularly impressive since the exposure to lead was particularly high being 1090 ppm in the drinking water from parturition to

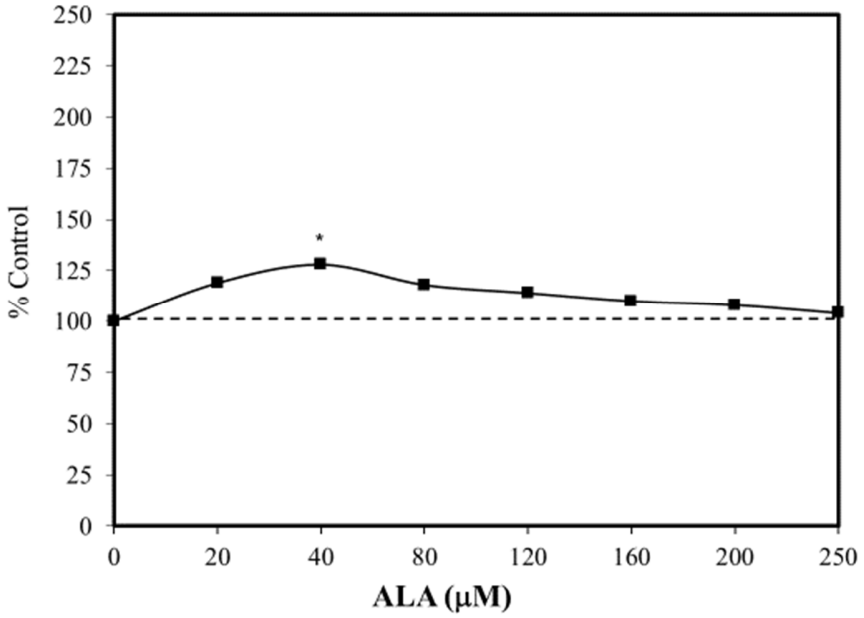


Figure 19. Effects of alpha lipoic acid (ALA) on viability (MTT assay) in isolated rat microchondria (modified from: Mozaffarian et al., 2022) *P= ≤ 0.05

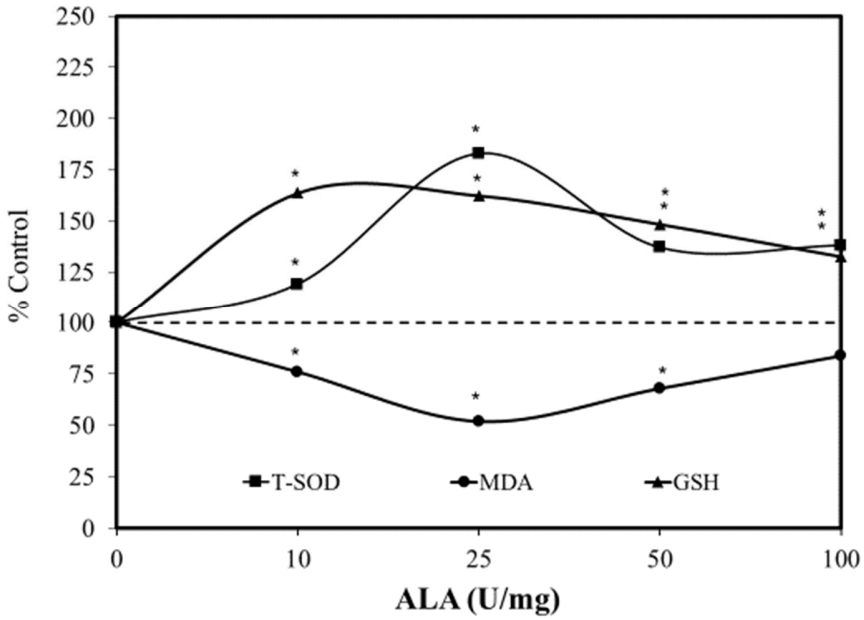


Figure 20. Effects of alpha lipoic acid (ALA) on the brain of chronically lead exposed rats (modified from: Wang et al., 2008) *P= ≤ 0.05

weaning. Lead exposure ceased at the end of weaning, at which time the ALA treatment was initiated. This protective effect may be framed within a post conditioning hormetic context.

10. AQUACULTURE

While there has been substantial research demonstrating that ALA induced hormetic dose responses in a broad range of human-relevant biomedical and experimental models, typically employing cellular models, there has been robust research with *in vivo* models in the area of aquaculture. These have focused on the capacity of ALA to enhance the health and commercial qualities of widely consumed shellfish and fish. The effect of ALA on agriculture-related species began in 2010 with the report of Zhang et al. (2010) on the effects of ALA on juvenile abalone, an important commercial shellfish. The studies focused on the antioxidant potential of ALA that was initially reported by Packer et al. (1995). In their study Zhang et al. (2010) evaluated the effects of ALA over a broad dosage range (200-3000 mg/kg) on the growth and biochemical parameters of the hepatopancreas. There was a dose dependent enhancement of antioxidant properties (e.g., GSH, SOD) that were associated with an increase in weight gain with the optimal dosage for each parameter being 800 mg/kg. Each parameter showed an hormetic-biphasic dose response with a low dose stimulation and a high dose inhibition (Figure 21). The authors recognized the biphasic nature of the dose response by highlighting the need to clarify why ALA decreased the responses at the higher dosages.

The findings of Zhang et al. (2010) inspired a spate of follow-up investigations until the present time, using a broad range of biological models (Kutter et al., 2012, 2013; Xiong et al., 2022; Xu et al., 2019a,b; Zhang et al., 2010). Following the same basic research design as Zhang

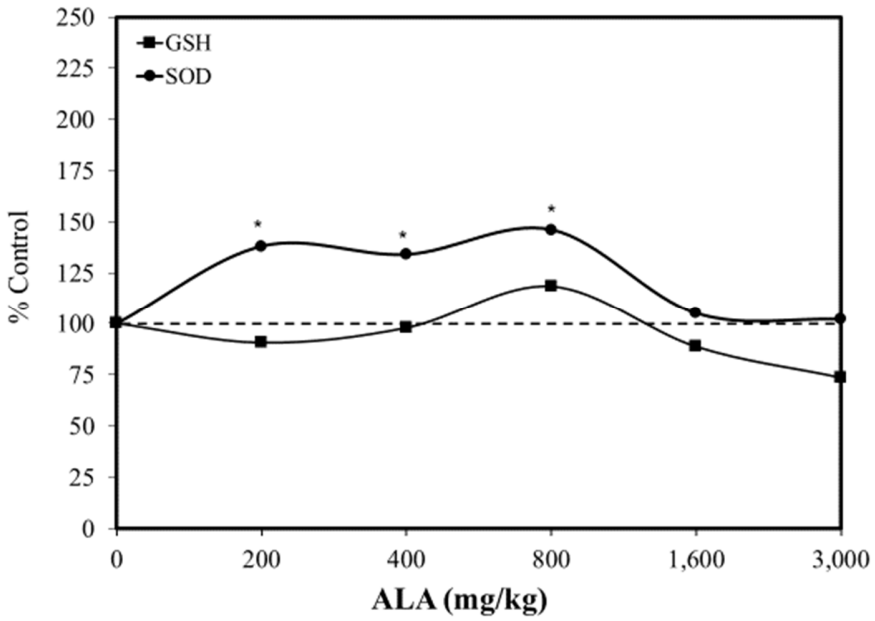


Figure 21. Effects of alpha lipoic acid (ALA) on hepatopancreas GSH and SOD levels of juvenile abalone, *Haliotis discus hannai* (modified from: Zhang et al., 2010) *P= ≤ 0.05

et al. (2010) these studies used some of the most prominent aquatic commercial species such as tilapia, grass carp, and sea bass as the models studied. In general, these studies employed 4-6 dosages ranging from about 200-4000 mg/kg in the test diet. The duration of these studies varied to some extent but typically were in the 56 to 70 day range using juveniles. Most studies used sample sizes/dose group in the range from 30 to 120 organisms. In general, all studies measured weight gain along with antioxidant-inflammatory parameters in multiple organs but with a strong focus on the liver and brain. Despite the wide range of aquatic species, each study displayed remarkably similar biphasic dose responses for the key parameters for growth and antioxidant enzyme activity, showing the low dose stimulation and high dose inhibition. Furthermore, there was also a strong consistency with respect to the maximum amplitude stimulation, with 131% being the median and with a median stimulation width of two-fold. Of particular note was the recent study of extensive ALA-induced hormetic effects on immune function in young grass carp and northern snakehead where the focus was on skin, kidney, spleen and brain for numerous parameters (e.g., TGFβ1, TGFβ2, IL-10, TNF-α and IL-1β) (Figures of these extensive data not shown) (Liu et al., 2018). Biphasic dose responses were also reported by Li et al. (2022b) on the effects of ALA on growth and antioxidant activity of the fish model *Channna argus* (Figure 22) (Li et al., 2022b). Of further interest is that Liu et al. (2018) showed the same types of hormetic responses within non-stressed fish as when they were challenged with skin hemorrhagic lesions. The ALA treatment reduced the occurrence of pathological effects in an hormetic biphasic manner. In this case as well, an excess dose of ALA enhanced the degree of injury. These collective findings with ALA show an enhancement of growth, the upregulation of adaptive mechanisms and the capacity to protect against some types of exogenous

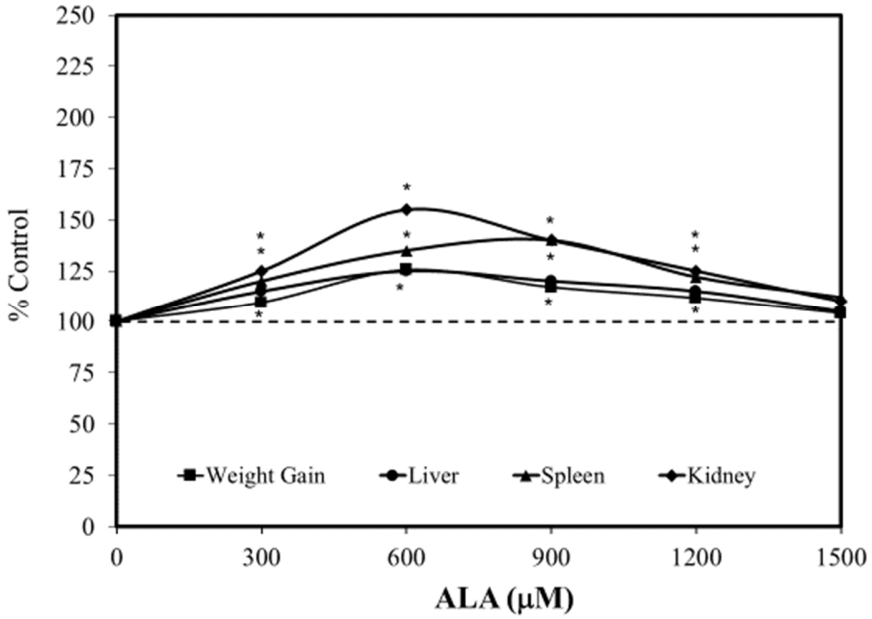


Figure 22. Effects of alpha lipoic acid (ALA) on growth and antioxidant activity of *Channa argus* (modified from: Li et al., 2022b) *P= ≤ 0.05

stresses/disease processes. For the most part these studies have been practically oriented showing a possible commercial path forward, whereby ALA might be used to enhance the growth and the adaptive capacity of a broad spectrum of aquaculture models. These studies have taken advantage of the more mechanistic cellular mammalian model studies and used these insights in more commercially oriented research activities. Further, the fact that ALA can be effective in the enhancement of immune function for several models suggests that this should be studied further in humans. Since ALA has already been approved for diabetic patients up to 1,200 milligrams per day it should be evaluated in clinical trials for its capacity to enhance immune function. Estimated plasma concentrations suggest that this dose would be in the range of hormetically active ALA for multiple endpoints discussed in this paper.

11. DISCUSSION

ALA was shown to induce protective effects in a broad range of tissues/cell types via hormetic dose response relationships. The most extensive studies have been practically oriented, being directed to the area of sperm preservation within the context of cryopreservation (Ibrahim et al., 2008; Asadpour et al., 2012). The ALA was effective across a broad spectrum of animal models using a range of experimental protocols. These findings were consistent with other antioxidants such as BHT (Asadpour et al., 2012; Khumran et al., 2015, 2017), alpha tocopherol (Ullah et al., 2019) and glycine (Nazif et al., 2022). Similar protective effects were also reported for human sperm with ALA within the context of assisting infertile couples achieve successful conception (Ibrahim et al., 2008).

Since ALA has been approved for the treatment of humans with diabetic neuropathy and nephropathy, researchers have directed attention to experimental conditions in which elevated glucose levels may induce adverse effects in the kidney (Leppert et al., 2017), heart (Yao et al., 2012) and brain cells (Li et al., 2022a; Najafi et al., 2015). These four studies used different cell types with each displaying their hormetic maximum responses within the 50-100 μM concentration range (i.e., podocytes, cardiomyocytes, corneal epithelial cells, and PC12 cells). Mechanistic evaluations in the diabetes-high glucose concentration studies indicate that ALA upregulated antioxidant enzymes in PC12 (Najafi et al., 2015) and corneal epithelial cells (Li et al., 2022a). In cardiomyocytes ALA was shown to activate the ERK1/2 pathway (Yao et al., 2012). Use of pathway specific blockers abolished the protective effect. The protective concentration activated the ERK1/2 pathway via the induction of a modest rise in ROS. Several studies with ALA induced hormetic effects in neuronal cells. These included the use of SH-SY5Y cells in which AGEs (Niu et al., 2018) and Aβ induced toxicities (Collins et al., 2023) were prevented (Figure 12). The ALA decreased the production of NO and ROS at low concentrations and enhanced the GSH content and glutamine synthetase activity in a hormetic fashion. ALA upregulates the expression of Nrf2-mediated antioxidant genes and peroxisome proliferation activated receptors-regulated genes to enhance the antioxidant defense systems (Golbidi et al., 2011). These collective findings show a consistent anti-inflammatory response at lower ALA concentrations while becoming pro-inflammatory at higher concentrations. ALA was also hormetic in other biological systems, including the skin, where it enhanced the viability of human melanocytes sites while showing the potential to act as a depigmenting agent (Martinez-Gutierrez et al., 2014) ALA showed the potential to prevent toxicity from elevated levels of environmental contaminants, such as arsenic Mozaffarian et al., 2022) and lead (Wang et al.,

2008). Of interest in this context is that the Mitrea et al. (2018) study suggested that the ALA might be used as a dietary supplement to protect against cosmic radiation during commercial flights based upon experimental studies with mice (Manda et al., 2007) in which it protected against radiation induced damage to the brain, kidney, testis, and liver. However, the ALA dosing was via IV and much higher (200 mg/kg) than that approved for human dosing.

There have been suggestions in the literature that ALA may be useful in muscle recovery within the context of competitive sports. For example, ALA reduced the serum concentration of creatine kinase following a 90 minute load, suggesting a protective effect against muscle damage (Morawin et al., 2014). A follow-up study by Isenmann et al. (2020) confirmed that in a six-day human training double-blind study, modest reduction of muscle damage and inflammation occurred in the ALA group (300 mg ALA in two doses; the first dose given two hours before while the second dose was given one hour immediately after the training episode). The authors suggested that ALA supplementation during intense training period can prevent muscle damage and inflammation and enhance recovery. It is hoped that future studies would clarify the impact of ALA on human performance in such studies using a broader dose range.

It is important to note that multiple ALA-induced hormetic effects with different cell types were optimized at concentrations that are readily achieved with clinically applied doses (600 – 1200 mg/day) in the *in vitro* systems. This represents an important observation and should be useful in developing future clinical research strategies for assessing a broad range of hormetic hypotheses in human subjects.

There has been considerable interest in assessing whether ALA might have the potential to slow the onset and progression of AD. Several reports with human subjects receiving 600 mg/day for up to four years appeared to markedly slow the progression of the disease. Supportive

studies in animal models for memory impairment showed significant improvement in a limited duration experiments (Farr et al., 2012). That ALA might ameliorate the effects of AD is generally supported by its capacity to affect multiple dimensions of the AD disease process (e.g., enhance acetylcholine production, accelerate clearance of Aβ plaques, and hence GSH content) suggests that ALA may affect its beneficial effects via the activation of hormetic mechanisms. The evidence for this perspective supports the need for follow-up research in both animal models and clinical trials. In contrast to many types of dietary supplements ALA has already been approved for the treatment of diabetic neuropathy and nephropathy, the dose being in the 600-1200 mg/day range. However, consumption of ALA via the diet is likely to be extremely modest as its presence in fruits and vegetable is limited. This condition suggests that there may be interest in ALA as a dietary supplement. Further, research is needed to better clarify the potential range of ALA applications (e.g., auditory) (Koo et al., 2016), (e.g., memory) (Hager et al., 2001) enhancement in humans which appear capable of affecting numerous biological systems.

12. FUNDING

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13. CONFLICT OF INTEREST

G. Dhawan is employed by Stantec (ChemRisk), a consulting firm that provides scientific support to the government, corporations, law firms, and various scientific/professional organizations. All other authors declare no conflict of interest.

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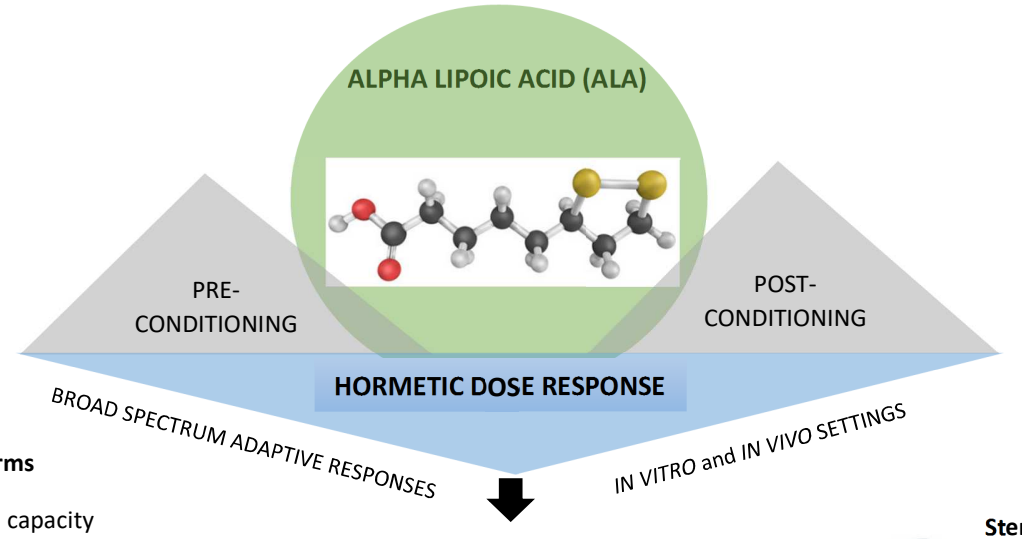
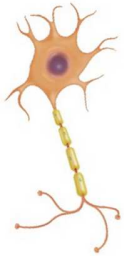
Alpha Lipoic Acid (ALA) induced broad spectrum protective and adaptive effects via hormetic dose response



Human and Animal Sperms

- Enhances functional capacity
- Upregulation of anti-oxidant capacity

Neuroprotection

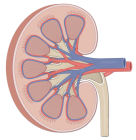


- PC12 cells – prevents apoptosis and restoration of anti-oxidant enzymes
- Glutamate toxicity – increases glutamate uptake and GSH content
- Alzheimer's disease – prevents Aβ protein aggregation and reduced Aβ fiber formation
- Hearing loss – prevents auditory damage induced by anti-biotics or chemotherapy

Corneal Cells

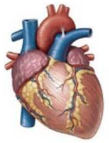
- Blocks AGEs (advanced glycation end products) induced oxidative stress on corneal cells in hyperglycemic environment

Renal Podocytes



- Enhances viability of podocytes in hyperglycemic environment

Myocardial Cells



- Prevents glucose oxidase induced injury

Stem Cells

- Promotes hematopoiesis
- Stimulates production of endothelial cells

Skin – Melanin Pigment

- Role in depigmentation – inhibits the expression of melanogenesis inducing tyrosinase enzymes

Arsenic, Lead

- Prevents arsenic induced mitochondrial toxicity
- Reverses lead induced oxidative stress