IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 15, Issue 2 Ser. II (February 2021), PP 01-09
www.iosrjournals.org

Influence of Vitamin E and Selenium on Reproductive Hormones and Lipid Peroxidation Levels in Lead-induced Toxicity in Female Wistar Rats.

Onitsha, Enebrayi N[1*] and Okutu, Jackson B[2].
[1*2]*The Department of Medical Laboratory Science, Faculty of Basic Sciences, College of Health Science, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria.*
**Corresponding Author: Onitsha Enebrayi Nelson. Department of Medical Laboratory Science, Faculty of Basic Sciences, College of Health Science, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa State, Nigeria.*

Abstract
Lead (Pb^{2+}) is a toxic metal in the environment and could adversely alter the functions of the female reproductive hormones: follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone and prolactin (PRL) which could lead to hormonal imbalance and consequently infertility. Infertility is a global health problem and a socially destabilizing condition. This study is aimed at investigating the antioxidant effect of vitamin E and/or selenium on FSH, LH, progesterone and PRL, and malondialdehyde (MDA) in lead exposed female wistar albino rats. A total of twenty-five rats were divided randomly into five groups of six rats each. Group A served as control and were administered 10mL/kg b wt distilled water. Group B were treated only with lead and received 250mg/kg b wt of lead acetate in drinking water. Group C received 250mg/kg b wt lead acetate and 600mg/kg b wt vitamin E. Group D received 250mg/kg b wt lead acetate and 0.5mg/kg b wt selenium. Group E received 250mg/kg b wt lead acetate and 600mg/Kg b wt vitamin E and 0.5mg/Kg b wt selenium. All treatments were by oral gavage and lasted for a period of six weeks. After the last day of treatment, the animals were sacrificed and blood samples were collected for determination of lead (Pb^{2+}), reproductive hormones and lipid peroxidation product, malondialdehyde (MDA). There was a statistically significant ($P<0.05$) reduction in the levels of LH, FSH, progesterone and PRL and a non-significant increase in lipid peroxidation in the lead acetate intoxicated rats (group B) compared with the control. There was a statistically significant ($P<0.05$) elevation of LH, FSH, PRL in groups C, D and E compared with the group treated only with lead. Progesterone was non-significantly and significantly reduced in group C and E respectively. However, progesterone was significantly ($P<0.05$) elevated in group D compare with the lead treated group B. The MDA concentration of groups C, D and E rats were significantly ($P<0.05$) reduced compared with group B. In conclusion, vitamin E, selenium and the combination of vitamin E and selenium have a protective effect on the reproductive hormone levels and lipid peroxidation.
***Keywords**: Lead Acetate, Vitamin E, Selenium, Reproductive Hormones, Oxidative stress, lipid peroxidation.*

Date of Submission: 03-02-2021 Date of Acceptance: 18-02-2021

I. Introduction

The endocrine system regulates the body's metabolism and functions including the reproductive hormones. The system consists of endocrine glands that act on their target organs through cognate receptors. The targets are in mainly endocrine organs that secrete hormones acting on the next level and also inhibiting the upper level via a negative feedback mechanism (1). Reproductive function is specifically under hormonal control and involves the hypothalamus in the brain, the pituitary gland that connected to the hypothalamus and the gonads (ovary in females). Thus, the hypothamic – pituitary – ovarian (HPO) axis plays a central role in the hormonal regulation of the female reproductive system (2). The HPO axis works collectively as to allow for reproduction by means of a cyclic production of gonadotropic and steroid hormones. This cycle is well regulated in order to select a dominant follicle for ovulation and as well as priming the endometrium for implantation. This complex regulation can be negatively impacted on when pathologies occur within any junction of the HPO axis (3) or when the HPO axis is exposed to endocrine disruptors (4,5). The ovary plays a vital role in the production of steroid hormone necessary for follicullar development and oocyte maturation (3). This process is regulated by the hypothalamic control of two gonadotropic hormones; follicle stimulating hormone (FSH) and leutenizing hormone (LH). While FSH is responsible for gametetogenic activity of the germinal epithelium, LH regulates secretory activities of leydig cells in males, and induces ovulation and formation of ovarian corpus in

females. Both gonadotropins influence oestrogen secretion from the ovary, and that has both inhibitory and, before ovulation, stimulatory effect on GnRH neurons and pituitary (1).

The HPO axis could be exposed to chemicals that have adverse effect on its regulatory functions. These so called "endocrine disrupting chemical substance" (EDCs) are exogenous chemical substances that impact adversely on the organism or the organism's progeny secondary to changes in endocrine function or action (6,7,8). They can interrupt with the syntheses, secretion, transport, binding or elimination of hormones in the body (9) and also interrupt with steriodogenesis and metabolism of hormones (7,8). The mechanism of action of these endocrine disruptors includes mimicking natural hormones, lock or binds on to a receptor within a cell, inhibiting the action of hormones and/or alters the normal regulation. Endocrine disruptors (ED) can reduce fertility; produce erectile dysfunction, abnormal sexual development, alteration in pituitary and thyroid gland function (10,11, 12).

Lead (Pb^{2+}) is a heavy metal and is categorized as one of the endocrine disruptors and induces modification of neurotransmitters in the central nervous system (CNS) and impairs the hypothalamic release of gonadotropin–releasing hormones (9 Bolawa et al, 2014). It is reported that lead (Pb^{2+}) is a powerful disruptor of adrenal steroidogenesis, inhibiting synthesis of progesterone and 17- hydroxyprogesterone. Testosterone and 17β oestrodiol are also inhibited when exposed to high levels of lead (Pb^{2+}) (13). Lead (Pb^{2+}) adversely affects the pituitary-hypothalamus axis, and the balance of gonadotropin (14). Lead is naturally occurring in the earth crust. Because of its widespread anthropogenic use, lead is ubiquitous in the environment. It is found in as a contaminate in consumer product, cosmetics, drinking water, food, natural products, therapeutic products, tobacco, environmental media including house dust and air (10).

Lead (Pb^{2+}) has been found to produce a wide range of toxic-biochemical effects involving biochemical activities (15,16). It is associated with altered sterodogonesis, decrease gonadotrophin binding and serum gonadotrophin levels in rats (17,18) and is associated with altered follicular growth and maturation in mice (19). Exposure to lower level of lead has been shown to reduce pituitary responsiveness to hypothalamic stimulus (20) thereby, altering the hormone production and subsequent circulation. Pollack and coworkers in one study reported that the geometric mean of lead (Pb^{2+}) increases with increase in mean progesterone. They reported that environmentally relevant levels of metals, lead (Pb^{2+}) inclusive are associated with modest changes in reproductive hormone levels in healthy and premenopausal women (21). Krieg and Feng (22) examined the association between blood lead level (BLL) and serum follicle stimulating hormones (FSH) and luteinizing hormone (LH) in women age 35 – 60 years and observed that as the blood lead level increased, the serum FSH concentration increases in pre-and postmenopausal women and women with both ovaries removed. Also, the concentration of LH increased as blood lead level increased in the postmenopausal women and women who had both ovaries removed. Lead (Pb^{2+}) may act directly or indirectly on the ovaries or other parts of the body and cause alteration in the level of FSH and LH. Gustafson et al, (23) reported that occupational lead (Pb^{2+}) exposure resulted in lower plasma levels of luteinizng hormones, follicle stimulating hormones, and cortisol, and a decrease in plasma selenium. In primates, prolonged expose to lead blocks ovarian and luteal function by reducing progesterone, luteinizing hormones and follicle stimulating hormone level (24).

The mechanism of lead-induced toxicity has been linked to oxidative stress (25). Oxidative stress results from the excessive production of reactive oxygen species (ROS) and the inability of the body's antioxidant defense system to mop up the excess. Lead (Pb^{2+}) induced oxidative stress has been implicated in endocrine disorders affecting the HPO axis (4). Antioxidants regulate the overproduction of reactive oxygen species (ROS). It therefore implies that antioxidants may play a vital role in cushioning and/or protect lead (Pb^{2+}) poisoning (26). The protective mechanism in animal is in the form of antioxidant nutrients, vitamins and enzymes. The body's defense mechanism depends on dietary intake of antioxidant vitamins and minerals. Vitamin E is an antioxidant vitamin whereas selenium is antioxidant mineral. Antioxidant such as vitamin E acts to protect the cells from the effect of free radicals, which are potentially damaging by-product of energy metabolism or from environmental exposure like radiation (27). Free radicals can damage the cells and contribute to development of cardiovascular disease, cancer, tissue injury in liver, (28, 29).

Vitamin E is able to interact with oxidizing radicals directly (30) and limit the production of free radicals. Vitamin E has been shown to play a role in immune function, in DNA repair, and other metabolic processes (31). Vitamin E functions to prevent membrane lipids from damage (32). Vitamin E can be obtained from many food sources, such as vegetable, oils, nuts, green leafy vegetable and fortified cereals (31). Vitamin E is a unique antioxidant [33] that is known to prevent reproductive disease associated with oxidative stress (34). Vitamin E interacts with oxidizing radicals and terminates the chain reaction lipid peroxidation and unsaturated membrane lipids due to its oxygen scavenging ability. It has been reported that vitamin E reduce the oxidative stress induced by lead or a toxic compound (35).

Selenium is an essential trace mineral element and a component of the cytosolic enzyme glutathione peroxidase which form part of the cell's antioxidant defense system (36,37). It plays an important role in preventing cells against oxidative damage by expressing selenoprotein genes and via anti-inflammatory

mechanism (38). Chen et al., (39) demonstrated that, selenoproteins, through their antioxidant properties, help to eliminate reactive oxygen species induced by metals. Kaneko, (40) reported that selenium facilitates the action of vitamin E in reducing peroxy radicals. It has also been demonstrated to have detoxification potentials on various heavy metal including lead (41). Selenium (Se) is considered as one of the most essential antioxidants (42a) as it plays an essential role in female reproductive function. It is implicated in determining the follicle growth, maturation, and dominance in both cows and women (43b) and in follicle dominance; protecting the dominant follicle from increasing levels of reactive oxygen species (ROS) (42a,43b). As sperm antioxidant, selenium protected its motility and fertility (44**).** Selenium is described as a much more potent anti-oxidant than vitamins E, C and A, beta-carotene, and much more toxic.

Vitamin E and selenium are essential nutrients known to have complementary biological functions as antioxidants to minimize cellular damage caused by endogenous peroxides (45). While selenium destroys a peroxide before it attacks the cell membrane (46), vitamin E acts within the cell membrane to prevent the formation of fatty acid hydro-peroxidation (47). Selenium and vitamin E work synergistically as it supports the activity of vitamin E in limiting oxidation of fat. However, there is paucity of literature on the influence of co-treatment with vitamin E and selenium on female reproductive hormone level in lead-induced toxicity in rats. Therefore, the present study is designed to investigate the possible preventive effect of simultaneous administration of vitamin E and selenium on the hormone levels in lead intoxicated rats.

II. Materials And Methods

Materials

Five hundred grams (500g) of Lead acetate of 99% purity with LOT #L117921504, DL-α- tocopherol acetate (99%) purity with LOT#L182801601 and anhydrous sodium selenite (98%) purity with LOT#Lmo125A1708 manufactured by LOBA CHEMIE PVT LTD, Mumbai India were procured/purchased from a reputable chemical and laboratory reagent supplier, Effective chemical and Laboratory reagent Ltd, Melford Okilo Rd,Yenagoa.

Animal treatment and Experimental Design

All procedures for animal handling and treatments were approved by the Ethical Committee Office of the department of pharmacology Niger University, (Amasssoma, Bayelsa state). A total of 25 female adult Wistar albino rats 7 - 8 weeks old and weigh between, 150-210 g was used in the present study. They were allowed to acclimatize for 2 weeks prior to the experiment. The rats were maintained under a light/dark cycle (12-12 h) and had access to food and water *ad libitum.* The rats were randomly divided into five groups (n=6/group) as follows:

Group A: Control. This group of rats received feed and water *ad libitum*

Group B: lead acetate. This group of rats received rat feed and were gavaged with lead acetate (250mg/kg body weight/day in drinking water) (48).

Group C: Lead acetate +**Vitamin E.** The rats in this group received rat feed and were gavage with lead acetate (250mg/kg body weight/day in drinking water) and vitamin E (600mg/kg body weight/day in Tween) (49).

Group D: Lead acetate + **Selenium (Se).** The rats received standard rat feed and water and were gavage with lead acetate (250mg/kg body weight/day in drinking water) and Selenium (0.5mg/kg body weight/day in drinking water) (50).

Group E: Lead acetate +**Vitamin E** + **Selenium.** The rats received rat feeds and were gavage with lead acetate (250mg/kg body weight/day) + Vitamin E (600mg/kg body weight/day) + Selenium (0.5mg/kg body weight/day).

Route and duration of administration: Lead acetate, vitamin E and selenium were administered orally by gastric gavage once daily for six consecutive weeks. At the end of the experiment, the rats were anaesthetized by inhalation with diethyl ether and then sacrificed. Blood samples were collected via cardiac puncture into heparinised and plain tubes. Blood in the heparinised tubes was used to estimate blood lead concentration. Blood in the plain tubes were allowed to cloth and centrifuged at 3000rpm for 10minutes to obtain serum. The clear serum was collected into another set of plain for estimation of Follicle stimulating Hormone (FSH), Luteinizing Hormone (LH), progesterone, prolactin and Malondialdehyde (MDA).

Analysis of Biochemical Parameters

Estimation of Blood Lead Levels.

Blood lead levels was measured by Atomic Absorption Spectrophotometer (AAS)

Estimation of Follicle stimulating hormones, luteinizing hormones, progesterone and prolactin levels.

Serum levels of Follicle stimulating Hormone (FSH), Luteinizing Hormone (LH), progesterone and prolactin were measured by ELISA technique using specific commercial kits. The assays were conducted according to the manufacturer's protocols.

Estimation of Malondialdehyde: MDA was determined by the method of Shah and Walker's, (51) using an auto analyzer spectrophotometer. Malondialdehyde in serum was separated and determined as conjugate with TBA. Serum proteins were precipitated by TCA and then removed by centrifugation. The MDA – TBA complex was measured at 534 nm (51). Briefly, 1.0ml reagent 1 (17.5% TCA), reagent 2 (70% TCA) and regent 3 (Thiobarbituric acid 0.6%) was added to 1.0ml of serum and mixed. The reaction mixture was incubated in boiling bath for 15 minutes, allowed to cool, and then let to stand at room temperature for another 20 minutes. Then the tubes centrifuged at 2000 rpm for 15 minutes and the supernatant layer was read at 534 nm. Distilled water was used for the blank. The concentration of MDA (nmol/ml) was calculated by using the following formula:

Concentration of the test= Abs (test) –Abs (blank) / 1.56 x 1000000

III. Results

Table 4.1 shows the hormonal profile and lipid peroxidation product MDA of the adult female rats studied. Data show significant reduction in FSH, LH, Progesterone and PRL in the lead acetate treated rats compared with their respective control. The MDA level was non-significantly elevated in the lead acetate intoxicated rats compared with the control. **Table 4.2** shows the hormonal profile and lipid peroxidation product MDA of the adult female rats exposed to lead acetate toxicity and treated with vitamin E. There was significant elevation in the level of FSH, LH, PRL and a nonsignificant reduction in the progesterone level compared with the control. **Table 4.3** shows the hormonal profile and lipid peroxidation product MDA of the adult female rats exposed to lead acetate toxicity and treated with selenium. There was significant elevation in the level of FSH, LH, PRL, Progesterone and a nonsignificant reduction in the MDA level compared with the control.

Table 4.4 shows the coadministration of vitamin E and selenium on some hormonal parameters and MDA in the lead treated rats. There was a significant elevation in the levels of FSH and LH compared with the positive control. Also, progesterone and prolactin showed a significant reduction compared with the positive control. MDA was statistically significant reduction compared with the positive control.

Table 4.1: Toxicological Assessment of Oral Lead Poisoning on Some Hormonal Parameters and Lipid Peroxidation Product of Adult Albino Rats Studied

Parameters (Units)	GP A	GP B	*t* statistic	*p* value	Remark
FSH (mIU/mL)	8.71 ± 0.28	8.27 ± 0.03	3.51	.024	S
LH (mIU/mL)	8.28 ± 0.04	7.23 ± 0.04	41.15	.000	S
PROG (nmol/L)	18.23 ± 1.22	14.22 ± 1.38	4.87	.001	S
Prolactin (ng/mL)	6.34 ± 0.23	6.02 ± 0.04	3.08	.015	S
MDA (nmol/L)	0.04 ± 0.03	0.07 ± 0.01	-2.07	0.100	NS

Key: GP A = Negative Control, GP B = Pb treated, NS = Not Significant, S = Significant

Table 4.2: Effect of Vitamin E Treatment on Some Hormonal Parameters of Oral Lead Induced Poisoning in Adult Albino Rats Studied

Parameters (Units)	GP A	GP B	GP C	*F* statistic	p value	Remark
FSH (mIU/mL)	$8.71 \pm 0.28^{\alpha}$	$8.27 \pm 0.03^{\beta}$	8.84 ± 0.23	10.10	.003	S
LH (mIU/mL)	$8.28 \pm 0.04^{\delta}$	$7.23 \pm 0.04^{\gamma}$	$7.56 \pm 0.15^{\varepsilon}$	168.62	.000	S
PROG (nmol/L)	18.23 ± 1.22	14.22 ± 1.38	7.24 ± 3.84	3.62	.059	NS
Prolactin (ng/mL)	$6.34 \pm 0.23^{\mu}$	$6.02 \pm 0.04^{\zeta}$	6.28 ± 0.09	7.01	.010	S
MDA (nmol/L)	0.04 ± 0.03	$0.07 \pm 0.01^{\ddot{o}}$	0.03 ± 0.00	6.17	.014	S

Key: GP A = Negative Control, GP B = Pb^{2+} treated, GP C = Vitamin E treated, NS = Not Significant, S = Significant. All post hoc testing were done using Bonferroni multiple comparison. $^{\alpha}$ Significant difference observed in the FSH concentration between GP A and GP B, $p < .05$. $^{\beta}$ Significant difference observed in the FSH concentration between GP B and GP C, $p < .01$. $^{\delta}$Significant difference observed in the LH concentration between GP A and GP B, $p < .001$. $^{\gamma}$ Significant difference observed in the LH concentration between GP B and GP C, $p < .001$. $^{\varepsilon}$Significant difference observed in the LH concentration between GP C and GP A, $p < .001$.$^{\mu}$Significant difference observed in the Prolactin concentration between GP A and GP B, $p < .05$.$^{\zeta}$Significant difference observed in the Prolactin concentration between GP B and GP C, $p < .05$. $^{\ddot{o}}$Significant difference observed in the MDA concentration between GP B and GP C, $p < .05$.

Table 4.3: Effect of Selenium Treatment on Some Hormonal Parameters and lipid peroxidation product of Oral Lead Induced Poisoning in Adult Albino Rats Studied

Parameters (Units)	GP A	GP B	GP D	*F*-statistic	*p* value	Remark
FSH (mIU/mL)	$8.71 \pm 0.28^{\alpha}$	8.27 ± 0.03	8.54 ± 0.04	9.16	.004	S
LH (mIU/mL)	$8.28 \pm 0.04^{\beta}$	$7.23 \pm 0.04^{\delta}$	$7.76 \pm 0.17^{\gamma}$	130.80	.000	S
PROG (nmol/L)	$18.23 \pm 1.22^{\varepsilon}$	$14.22 \pm 1.38^{\mu}$	$16.28 \pm 0.50^{\zeta}$	16.56	.000	S
Prolactin (ng/mL)	$6.34 \pm 0.23^{\ddot{o}}$	$6.02 \pm 0.04^{\emptyset}$	6.64 ± 0.13	7.15	.009	S
MDA (nmol/L)	0.04 ± 0.03	0.07 ± 0.01	0.04 ± 0.04	3.91	.051	NS

Key: GP A = Negative Control, GP B = Pb treated, GP D = Selenium treated, NS = Not Significant, S = Significant. All post hoc testing were done using Bonferroni multiple comparison. $^{\alpha}$ Significant difference observed in the FSH concentration between GP A and GP B, $p< .05$. $^{\beta}$ Significant difference observed in the LH concentration between GP A and GP B, $p< .001$. $^{\delta}$Significant difference observed in the LH concentration between GP B and GP D, $p< .001$. $^{\gamma}$ Significant difference observed in the LH concentration between GP D and GP A, $p< .001$. $^{\varepsilon}$Significant difference observed in the Progesterone concentration between GP A and GP B, $p< .001$.$^{\mu}$Significant difference observed in the Prolactin concentration between GP B and GP D, $p< .05$. $^{\zeta}$Significant difference observed in the Progesterone concentration between GP D and GP A, $p< .05$. $^{\ddot{o}}$Significant difference observed in the Prolactin concentration between GP A and GP B, $p< .05$.$^{\emptyset}$Significant difference observed in the Prolactin concentration between GP B and GP D, $p< .05$.

Table 4.4: Effect of Vitamin E + Selenium Treatment on Some Hormonal Parameters of Oral Lead Induced Poisoning in Adult Albino Rats Studied

Parameters (Units)	GP A	GP B	GP E	*F* statistic	*p* value	Remark
FSH (mIU/mL)	$8.71 \pm 0.28^{\alpha}$	8.27 ± 0.03	8.53 ± 0.13	7.72	.007	S
LH (mIU/mL)	$8.28 \pm 0.04^{\beta}$	$7.23 \pm 0.04^{\delta}$	$7.48 \pm 0.05^{\gamma}$	740.67	.000	S
PROG (nmol/L)	18.23 ± 1.22	14.22 ± 1.38	$12.52 \pm 4.30^{\varepsilon}$	5.90	.016	S
Prolactin(ng/mL)	$6.34 \pm 0.23^{\mu}$	6.02 ± 0.04	$5.95 \pm 0.09^{\zeta}$	10.16	.003	S
MDA (nmol/L)	0.04 ± 0.03	0.07 ± 0.01	$0.03 \pm 0.00^{\ddot{o}}$	6.17	.014	S

Key: GP A = Negative Control, GP B = Pb treated, GP E = Vitamin E + Selenium treated, NS = Not Significant, S = Significant. All post hoc testing were done using Bonferroni multiple comparison. $^{\alpha}$ Significant difference observed in the FSH concentration between GP A and GP B, $p< .01$. $^{\beta}$ Significant difference observed in the LH concentration between GP A and GP B, $p< .001$. $^{\delta}$Significant difference observed in the LH concentration between GP B and GP E, $p< .001$. $^{\gamma}$ Significant difference observed in the LH concentration between GP E and GP A, $p< .001$. $^{\varepsilon}$Significant difference observed in the Progesterone concentration between GP E and GP A, $p< .05$.$^{\mu}$Significant difference observed in the Prolactin concentration between GP A and GP B, $p< .05$. $^{\zeta}$Significant difference observed in the Prolactin concentration between GP E and GP A, $p< .001$. $^{\ddot{o}}$Significant difference observed in the MDA concentration between GP E and GP A, $p< .05$.

IV. Discussion

Human and animals are exposed to the heavy metal disruptor lead (Pb^{2+}) throughout their life time through industrial emissions, soil, car exhaust gases, and contaminated foods, (52), acid batteries, alloys, cable sheathing, pigments, and rust-proof (53). Environmental lead accesses the body via inhalation of airborne contaminated dust or ingestion of contaminated food and water into the digestive tract (54).

Lead (Pb^{2+}) is known to disturb the normal profile of reproductive hormones in animals, both at the hypothalamic, pituitary and gonodal levels (4). Lead adversely affects hypothalamus-pituitary axis, and the balance of gonadotropin (14). Normally in the brain, the hypothalamic gonadotropin releasing hormone (GnRH) neuron stimulate pituitary gonadotropins to secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH) which acts on the gonads (ovary in females). FSH and LH are either positively or negatively modulated by oestrogen and progesterone, depending on the concentration and period of exposure of the pituitary to these steroids (55). A feedback mechanism exists between the secretion of oestrogen and the release of gonadotropins from the hypothalamus (GnRH) and pituitary (FSH, LH) and is observed when there is an increase in the secretion of FSH and LH as in menopausal women and castrated men (56). Disruption of the hypothalamic pituitary ovarian (HPO) axis by endocrine disrupting chemical substances (EDCs) such as lead (Pb^{2+}) can impact adversely on the regulatory function of the GnRH and affect the levels of reproductive hormones. In the present study, FSH, LH, progesterone and prolactin levels were suppressed post administration with lead (Pb^{2+}) compared with the control. This finding was in tandem with the study by Frank et al 24 (1989) and Foster, (57). Frank and coworkers observed that in primate, chronic lead (Pb^{2+}) exposure obstruct ovarian and luteal function by reducing FSH, LH and progesterone levels while Foster, (57) reported that subclinical suppression of luteinizing hormone, follicle stimulating hormones, oestrogen and progesterone are associated with chronic lead exposure. The reduction in reproductive hormone levels in this study can be attributed to accumulation of lead (Pb^{2+}) in the hypothalamus and pituitary which could have disrupted the feedback mechanism that brings about hormone homeostasis. Normally, low progesterone level has a feedback control on the GnRH neuron to stimulate FSH and LH production thereby increasing the level of these hormones. Therefore, a low FSH and LH observed in this study are suggestive of the disruption or impairment of the feedback mechanism by the endocrine disruptor lead (Pb^{2+}). Also, a low prolactin in this study may be due to disruption of the feed-back mechanism as high prolactin has an inhibitory effect on LH and FSH secretion at the pituitary level. Studies have demonstrated that lead (Pb^{2+}) exposure disrupted the hormone feedback mechanism at the hypothalamic – pituitary level (58). The reductive hormone levels could also be due to impairment of GnRH neuron transmission caused by lead (Pb^{2+}). Chronic exposure of rats to low lead (Pb^{2+}) levels is known to cause a

reduction in the release of Ca^{2+} - dependent glutamate and γ-aminobutyric acid (GABA) in the hippocampus (59,60) resulting in dysfunction of the presynatic neuron signaling in the hippocampus (61). The inhibitory post synaptic currents (IPSCs) and excitatory post synaptic currents (EPSCs) both of which depend on glutamate and γ-aminobutyric acid (GABA) are also impaired by lead (Pb^{2+}). this leads to reduction in IPSCs and EPSCs thus indicating a deficit in glutamatergic and GABAergic neurotransmission systems. Lead can disrupt neurotransmission by inhibiting the neuronal voltage-gated calcium (Ca^{2+}) channels (VGCCs) (62). Thus, inhibition of presynaptic VGCCs may reduce the influx of Ca^{2+} which is required for fast release of vesicular neurotransmitter thus interfering with neurotransmission. The altered levels of the reproductive hormones could also be attributed to decreased fluidity of the pituitary membrane. Exposure to low lead level is known to decrease the fluidity of the pituitary membrane in rats, a scenario that that impair pituitary and receptor binding (63). In the present study the reduction in the level of progesterone post lead (Pb^{2+}) intoxication could also be attributed to increased activity of a progesterone – metabolizing enzyme progesterone-5β reductase (63) in liver and uterine homogenate in mice. This enzyme reduces progesterone to 5β reduced metabolites.

Animal studies have suggested that the adverse effect of lead (Pb^{2+}) can be reversed when lead (Pb^{2+}) is removed from the system (10). In the present study, treatment of lead intoxicated rats with vitamin E improved the levels of LH, FSH and prolactin compared with the lead treated control. This could be due to the reactive oxygen species (ROS) scavenging ability of vitamin E which may have improved the GnRH neuronal transmission and feedback mechanism restored. The antioxidant activities of vitamin E were reported following findings on its ability to scavenge reactive oxygen species (ROS) in cellular membranes (65).

Selenium treatment restores the levels of FSH, LH, progesterone and prolactin above that of the lead treated control. This is in line with Shen et al, (66) who reported that Selenium decreases serum lead and rectified reproductive hormones. The improved concentration of these hormones maybe due to enhancement of endogenous GSH activity by selenium. Selenium is known to enhance the availability of GSH, an intrinsic antioxidant that help in preventing lipid peroxidation and resultant cell damage (67).

Studies have revealed that antioxidants, in addition to their individual effects can interact synergistically to produce a sparing effect, such that one antioxidant protects another against oxidative destruction. Combining antioxidants to prevent cellular injury has proven to be more effective than using larger quantities of any single antioxidants (68). In the present study the co-administration of vitamin E and selenium improved the concentrations of FSH and LH significantly but did not elevate progesterone and prolactin compared with the lead treated control.

In part this is not consistent with the observation of Young and Lowe, (68) on combining antioxidants. There could be two possible explanations for this observation. Firstly, the proportion (ratio) and concentration in which the two antioxidants are combined may cause either antioxidant to be become antagonistic to the other. Secondly, the administered lead depleted the endogenous selenium stores such that the supplemented selenium was not enough to have a positive impact on the hormone levels. Gustafson et al (23) reported that occupational lead exposure resulted in a decrease in plasma selenium.

Studies have shown that chronic exposure to lead resulted in increased lipid peroxidation (69). Lipid peroxidation product MDA is a clinical marker of oxidative stress and is often used as a measure of tissue damage (70). Lead is absorbed into the bloodstream and accumulation in erythrocytes due to its high affinity for δ -aminolevulinic acid dehydratase (ALAD), the enzymes that catalyse the conversion of aminolevulinic acid (ALA) to porphobilinogen (PBG) (71,72). Lead displaces Zn^{2+} at the metal binding site in the enzyme and change the enzyme structure by binding to –SH groups. This results in the inhibition of δ -aminolevulinic acid dehydratase (ALAD) (73). Consequently, ALA accumulates and depresses haem synthesis, which stimulates the production of more ALA that accumulates in blood (74). ALA accumulation is linked to generation of reactive oxygen species (ROS) as well as lipid peroxides (75) which may lead to state of oxidative stress and a resultant damage to lipid, protein and DNA (67). The increased lipid perioxidation observed in this study is in conformity with the finding of (69). which showed increase MDA levels in the lead intoxicated rats compared with the control. The increased lipid peroxidation observed in this study may be due to inhibition of δ-aminolevulinic acid dehydratase (76) resulting in the accumulation of δ-aminolevulinic, a potential source of free radical (77). According to Lawton and Donaldson (78), lead (Pb^{2+}) induced arachidonic acid elongation might be responsible for the enhanced lipid peroxidation in the membrane, which may be reflected in the serum.

Vitamin E therapy on the lead (Pb^{2+}) exposed rats ameliorated lipid peroxidation compared with the lead intoxicated control. The reduction in MDA concentration by vitamin E administration was in agreement with the study by (79) which reported that vitamin E provide fortification against oxidative stress caused by lead poisoning in rats exposed to lead. The reduction in lipid peroxidation could be attributed to the scavenging of peroxyl radicals and inhibition of the free radical chain reaction of lipid peroxidation (80). Vitamins E reduce the production of reactive oxygen species (ROS) through their "scavenging", "quenching" and "mopping up" properties (81).

Selenium has also been demonstrated to reduce the oxidative stress in lead toxicity (82). In the present study, selenium administration reduced MDA level in the lead intoxicated rats compared with the control. Our finding is in conformity with previous studies (83,84). The reduced level of MDA in selenium therapy may be due to its antioxidant properties to form a complex with lead (85).

Combination of selenium and other antioxidants has been demonstrated to reduce oxidative stress in animals (86,36). It has been reported that the synergistic effects of antioxidants such as selenium and vitamin E is the most powerful in reducing ROS toxicity (87,88). Also, studies have demonstrated the synergism between vitamin E and selenium in counteracting free radical induced oxidative stress (35).

The combined administration of vitamin E and selenium in this study reduced lipid peroxidation. This is in agreement with a recent study by, (82) who showed that the supplementation of vitamin E and selenium reduces lipid peroxidation. The reduction of MDA level in this study could be attributed to the antioxidant properties of both vitamin E and selenium in scavenging of peroxyl radicals and inhibition of the free radical chain reaction which leads to lipid peroxidation (89).

V. Conclusion

The level of the productive hormones (FSH, LH, progesterone and prolactin) was negatively impacted upon by lead treatment. Vitamin E and selenium treatments alone or as a mixture restored levels of LH and FSH but did not have a restoring effect on progesterone and prolactin. The increased lipid peroxidation was ameliorated by Vitamin E and selenium treatments alone or as a mixture.

References

[1]. World Health Organization (2012). Endocrine disrupters and child health: possible developmental early effects of endocrine disrupters on child health. pp. 1 – 52.

[2]. Arrais, R. F., & Dib, S. A (2006). The hypothalamus – pituitary – ovary axis and type 1 diabetes mellitus: a mini review. *Journal of Human Reproduction*. Vol. 21. Issue 2. pp. 327 – 337.

[3]. Mikhael, S., Advaita, P., & Larisa, G. (2019). Hypothalamic – Pituitary – Ovarian Axis Disorders Impacting Female fertility. *Biomedicine*. Vol.7. Issue 5. pp. 2 – 9.

[4]. Ronis, M.J.J, Badger T. M, & Shema S. J. (1996). Reproductive toxicity and growth effects in rats exposed to lead at different periods during development. *Toxicology applied. Pharmacology*. Vol. 136. pp. 101 – 120.

[5]. Sokol, R. Z. (1987). Hormonal effects of lead in the male rat: mechanism of action. *Reproduction*. Vol. 37. pp. 1135 – 1138.

[6]. Saalu, I. C., & Osinubi, A. A. (2009). Environmental endocrine disruptor of testicular function. African Journal of Endocrinology and metabolism. Vol. 8. Issue 1. pp.13 – 23.

[7]. Sweeny, T. (2002). Is exposure to endocrine disrupting compounds during fetal/postnatal development affecting the reproductive potential of farm animals?. *Domestic Animal Endoccrinology*. Vol. 23. pp. 203- 209.

[8]. Sanderson, J. T. (2006). The steroid hormone biosynthesis pathway as a target for endocrine disrupting chemicals. *Toxicological sciences*. Vol. 94. pp. 3 - 21.

[9]. Bolawa, O. E., Gbenle, G. O., & Ebuchi, O. A (2014). Endocrine disruption by the consumption of fish (Tilapia oreochromis) from heavy metals polluted river sites and its reversal using zinc. *International Journal of Aquaculture.* Vol. 4. Issue 14. pp. 85 – 88

[10]. Duru, F.I, Osinubi A.A, Alebiosu C.O & Falana B.A. (2015). Anatomy of lead poisoning. *Research Journal of Health Science.* Vol 3. Issue 3. pp. 149 -162

[11]. Woodruff, T.K., & Walker, C. L. (2008). Fetal and early post-natal environmental exposure and reproductive health effects in female. *Fertility Sterility.* Vol. 89: pp. 47 – 51

[12]. Patel, S., Zhou, C., Rattan, S & Flaws, J. A. (2015). Effects of endocrine disrupting chemicals on the ovary. *Biology of Reproduction*. Vol. 93. pp. 20

[13]. Chikezie, I. C., Charles-Davies, M. A., Balogun, A.M., & Okoli, S. U. (2017). Effects of Endocrine Disrupting Heavy Metals on Pituitary and Gonadal Hormones in Normal Weight Automechanics in Ibadan, Nigeria. *Journal of Biomedical Research.* Vol.20: pp. 25 – 35.

[14]. Sharma, R., Qereshi, M., Mogras, S., & Panwar, K. (2012). Lead Induced Infertility in Swiss Mice and Role of Antioxidants. *Universal Journal of Environmental Research and Technology*. Vol. 2. issue 2. pp. 72 – 82.

[15]. Ademuyiwa , O., Arowolo, T., Ojo, D. D., Odukoya, O.O., Yusuf, A.A & Akinhami, T.F. (2002). Lead levels in blood and urine of some residents of Abeokuta, Nigeria. *Trace Elements and Electrolytes*. Vol. 19. pp. 63 – 69.

[16]. Sakai, (2000). Biomarkers of lead exposure. *Industrial Health*. Vol. 38. pp. 127 – 142.

[17]. Priya, P. N., Pillai, A., & Gupta, S. (2004). Effect of simultaneous exposure to lead and cadmium on gonadotropin binding and steroidogenesis on granulose cells: an invitro study. *Indian Journal exp. Boil*. Vol. 42. pp. 143 – 148.

[18]. Nampoothiri, L. P., & Gupta, S. (2006). Simultaneous effect of lead and cadmium on granulose cells: a cellular model for ovarian toxicity. Reproductive Toxicology. Vol. 21. pp. 179 -185.

[19]. Junaid, M., Chowdhuri, D. K., Narayan, R., & Saxena, D. K. (1997). Lead Induded changes in ovarian follicular development and maturation in mice. *Journal Toxicology environmental Health*. Vol. 50. Issue 1. pp. 31 – 40.

[20]. Camoratto, A., White, L.M., Lau, Y.S., Ware, G.O., Berry, W.D., & Morcarty, C.M. (1993): Effect of exposure to low level lead on growth and growth hormone release in rat. *Toxicology.* Vol. 83. Issue 3: pp. 101 – 114.

[21]. Pollack, A.Z., Schisterman, E. F., Goldman, L.R., Munford, S. L., Abert, P. S., Jones R. L, et al (2011). Cadmium, lead and mercury in relation to reproductive hormones and an ovulation in premenopausal women. *Environmental Health perspective*. Vol.19. pp. 1156-1161.

[22]. Krieg, E. F. J. & Feng, H. A. (2011). The relationship between blood lead levels and serum follicle stimulating hormone and luteinizing hormone in the National Health and Nutrition Examinaiton Survey 1999-2007. *Report Toxicology*. Vol. 32. pp. 277-285.

[23]. Gustafson, A., Hender, P., Schiitz, A. and Skerfvings, S. (1989). Occupational lead exposure and pituitary function. *Internal Archive Occupational Environment Health*, vol. 61. Issue. 4: pp. 277 – 281.

[24]. Frank, P. A., Laughin, N. K., Dursche, D. J., Bowman, R.E and Meller, P. A (1989). Effect of lead on luteal function in Rhesus monkey. *Boil. Reproduction*. Vol. 41. Issue 16. pp. 1055 – 1062.

[25]. Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead: A review with recent updates. *Interdisciplinary Toxicology*. Vol. 5. Issue 2: pp. 47 – 52.

[26]. Gurer, HOzygunes, H., Saygin, E., and Ercal, I.N. (2004). Antioxidant effect of taurin against lead-induced oxidative stress. *Archives of environmental contamination and Toxicology*. Vol. 41. Issue 4: pp. 397 – 402.

[27]. Farell, P., and Roberts, R. (1994). Vitamin E: In Shila ME, Olson JA, Shike M, Ross AC, ed. Modern nutrition in health and disease 8th ed. Philadelphia, PA: Lea and Febiger, pp. 326 – 341.

[28]. Weiss, S. J.(1989). Tissue destruction by neutrophils. *New English Journal medicine*. Vol. 320: pp. 365 – 376.

[29]. Halliwell, B. (1994): Free Radicals, Antioxidants, and human diseases: Curiosity, caus,or consequence? *Lancet*, pp. 344– 721.

[30]. Jones, D. P., Kagam, V.E., Aust, S.D., Reed, D.J., & Oomaye, S. T. (1995). Impact of nutrients on cellular lipid peroxidation and antioxidant defense system. *Fundamental Applied Toxicology pharmacology*, Vol. 59: pp. 164 – 172.

[31]. U.S Department of Agriculture, Agriculture Research Service (2004). USDA National Database for standard Reference, Release 16 – 1. Nutrient Data LaboratoryHome Page, www. Avs. Usda.gov/ba/bhncr/ndl.

[32]. Frei, B. (1991). Ascorbic acid protects lipids in human plasma and low-density lipoprotein against oxidative damage. *American Journal clinical Nutrition*. Vol. 24. pp. 1113 – 1118.

[33]. Chow, C.K. (1991). Vitamin E and oxidative stress. Free radic. *Boil. Med*. Vol. 11. Issue 2. pp. 215 – 235.

[34]. Brigelius-Flohe, R., Kelly, F. J., Salonen, J.T., Neuzil., Zingg, J. M & AZZI, A. (2002). The European Perspective on vitamin E: current knowledge and future research. American Journal clinical Nutrition. Vol. 76. Issue. 4. pp. 703 - 716

[35]. Bansal A.K., Bansal, M., Soni, G., & Bhatnagar, D. (2005). Protective role of vitamin Epre-treatment on N-nitrodiethylamine induced oxidative stress in liver. *Chemical boiling Interaction*. Vol. 156. pp. 101- 111.

[36]. Kowluru, R. A., Engerman, R. L., and Kern, T. S (2000). Diabetes – induced metabolic abnormalities in my ocardium: effect of antioxidant therapy. *Free Radical Research*. Vol 31. pp. 67-74.

[37]. 37. Atroshi, F., Rizzo, A., & Biese, I et. al. (1999). Fumonism B1-induced DNA damage in rat liver and spleen : effects of pretreartment with coenzyme Q10, L-carnitine, α-tocopherol and selenium. *Pharmacological Research*. Vol. 40. Issue 6. pp. 459 - 467

[38]. Said, R.S., Sadr, A.M., Nada, A. S., & El-Demerdash. (2014). Sodium selenitetreatment restore long-lasting ovarian damage induced by irradiation in rats: impact on oxidative stress and apoptosis. Reproductive Toxicology. Vol. 43. pp. 85 – 93.

[39]. Chen, Z., Meng, H., Xing, G., Chen, C., Zhao, Y., Jia, G., Wang, T., Yuan, H., Ye, C., Zhao, F., Chai, Z., Zhu, C., Fang, X., Ma, B., Wan, L., 2006. Acute toxicological effects of copper nanoparticles in vivo. *Toxicology Letter*. Vol. 163. pp. 109–120.

[40]. Kaneko, J. J. (1989). Clinical Biochemistry of Domestic Animals, 4th edn, San Diego: Academic Press, Inc, USA.

[41]. Diplock, A T., Watkin., W. J. & Heurson, M. (1986). Selenium and heavy metals. *Annual Clinical Research*. vol. 18. pp. 55-60.

[42]. Ceko, M.J., Hummitzsch, K., Hatzirodos, N., Bonner,W.M., Aitken, J.B., Russell, D.L., Lane, M., Rodgers, R.J., & Harris, H.H. (2015a). X-Ray fluorescence imaging and other analyses identify selenium and GPX1 as important in female reproductive function. *Metallomics*, Vol. 7. pp. 71–82.

[43]. Ceko, M.J.; Hummitzsch, K., Bonner,W.M., Aitken, J.B., Spiers, K.M., Rodgers, R.J., & Harris, H.H. (2015b). Localization of the trace elements iron, zinc and selenium in relation to anatomical structures in bovine ovaries by X-ray fluorescence imaging. *Microsc. Microanal*., vol. 21. pp.695–705.

[44]. Qazi, I. H., Angel, C, Yang, H., Zoidis, E., Pan, B., Wu, Z., Ming, Z., Zeng, C-J., Meng, Q., Han, H., & Zhou, G*et* (2019). Role of selenium and selenoproteins in male reproductive function: a review of past and present evidences. Antioxidants (Basel). Vol. 8. Issue. 8. pp. 2-36.

[45]. Kolb D. (1997). Nutritional aspects of the importance, utilization, metabolism and the use of vitamin E and selenium in sheep. Berl. Munch. Tierar Ztl. Wschr. Vol. 110. pp. 178 – 184

[46]. Ebert – Duniig, R., Seufert, J., Schineider, D., Kohrle, D., Schurtze, N., & Jakob., F. (1999). Expression of selenoprotein in monocyte and macrophages-implications for the immune system. Med. Kein, Vol. 94. pp. 29 – 34.

[47]. Chow, C. K. (2001). In: Rucker, r., Suttle, J.W., McCormick, D. B., and Machlin, L.J, Editiors, 2001. Handbook of vitamins. Marcel Dekker, New York, pp: 165 – 196.

[48]. Suleiman, J. B., Eze, E. D., Momoh, I. J., Usman, W., Hedima, N. C., Zipele, H. M. & Isa, A. (2013). Ameliorative Effect of Vitamin C on Serum Liver Enzymes in Lead-Induced Toxicity in Wistar Rats**.** *Journal of Science*. Vol. 3. Issue. 1. pp. 188 - 192.

[49]. Ramah, A., Ragab, M. E., Nabila, M.A., & Elham A. E (2015). The effect of lead toxicity on male albino rats reproduction with ameliorate by vitamin E and pumpkin seeds oil *Benha Veterinary Medical Journal*, Vol. 28. Issue 1. pp. 43-52**.**

[50]. Kiełczykowska, M., Kocot, J., Lewandowska, A. Żelazowska, R. & Musik, I. (2015). The Protective Influence of Selenium on Oxidant Disturbances in Brain of Rats Exposed to Lithium. *Physiology Research*. Vol. 64. pp. 739-746.

[51]. Shah, J.K., and Walker's, A.M (1989). Quantitative determination of MDA. *Biochem. Biophys. Acta*. Vol. 11. pp. 207-211.

[52]. Gama, E.M., DaSilva, L.A., & Lemos, V.A. (2006). Preconcentration system for cadmium and lead determination in environmental samples using polyurethane foam/Me-BTANC, *Journal of Hazardous Materials*. Vol. 136: pp. 757–762.

[53]. Quinn, M.J., & Sherlock, J.C. (1999). The correspondence between U.K.; action level; for lead in blood and in water. *Food Addition Contaminant*. Vol. 7. Vol. 3. pp. 387-424.

[54]. Lamidi, I.Y., & Akefe, I.O. (2017) Mitigate Effects of Antioxidants in Lead Toxicity. *Clinical Pharmacology and Toxicology Journal*. Vol. 1. Issue 3. pp. 1– 9.

[55]. Fink, G. (1988). Gonadotropin secretion and its control. in: Knobil, E and Neil, J. D (eds). The Physiology of reproduction. New York: Raven Press. Vol.1. pp.1349 – 1377.

[56]. Carr, B. R (1998). Disorders of the ovaries and female reproduction tract. In: Wilson, J. D, Forster, D. W, Kronenberg, H. M, & Larsen (eds). Williams Textbook of Endocrinology. 9th edn. Sauders, New York. pp. 751 – 817.

[57]. Foster, W.G (1996). Lead and female reproductive function. *Food Chemistry Toxicology*. Vol. 34. Issue 9. pp. 927.

[58]. Sokol, R.Z. (1987). Hormonal Effects of Lead Acetate in the Male Rat: Mechanism of Action. Biology Reproduction. Vol. 37. pp. 1135-1138.

[59]. Lasley, S.M., & Gilbert, ME. (1996). Presynaptic glutamatergic function in dentate gyrus in vivo is diminished by chronic exposure to inorganic lead. Brain Research. Vol. 736. pp. 125-134

[60]. Xiao C, Gu Y, Zhou CY, Wang L, & Zhang MM. (2006). Pb2+ impairs GABAergic synaptic transmission in rat hippocampal slices: A possible involvement of presynaptic calcium channels. *Brain Research.* Vol. 1088. pp. 93-100.

[61]. Braga, M.F, Pereira, E. F., & Albuquerque, E.X. (1999). Nanomolar concentrations of lead inhibit glutamatergic and GABAergic transmission in hippocampal neurons. *Brain Research*. Vol. 826. pp. 22-34

[62]. Peng S., Hajela R. K., & Atchison, W. D. (2002). Characteristics of block by Pb2+ of function of human neuronal L-, N-, and R-type Ca2+ channels transiently expressed in human embryonic kidney 293 cells. *Molecular Pharmacology*. Vol. 62. pp. 1418-1430.

[63]. Pillai, A., Priya, L., & Gupta, S. (2002). Effects of combined exposure to lead and cadmium on the hypothalamic-pituitary axis function in proestrous rats. *Food and Chemical Toxicology*. Vol. 41. pp. 379 – 384.

[64]. Abdou, H. M., & Newairy, A. A. (2006). Hepatic and reproductive toxicity of lead in female rats and attenuation by flax seed lignans. *Journal medical Research Institute*. Vol. 27. pp. 295 – 302.

[65]. Esterbauer, H., Dieber-Rotheneder, M., Striegl, G., & Waeg, G. (1991). Role of vitamin E in preventing the oxidation of low-density lipoprotein. *American Journal Clinical Nutrition*. Vol. 53. pp. 314–321.

[66]. Shen, W., Chen J., Yin J., & Wang S.L., (2016). Selenium protects reproductive system and foetus development in rat model of geststional lead exposure *European Review for medical and pharmacological science*. Vol. 20. Issue 4. pp. 773-780

[67]. Patra, R. C., Rautray, A. K., & Swarup, D. (2011). Oxidative stress in lead and cadmium toxicity and its amelioration. *Veterinary Medicine International*. pp. 1-9.

[68]. (2001) Young, A.J & Lowe, G.M. (2001). Antioxidant and prooxidant properties of carotenoids. *American Journal of Archives of Biochemistry and Biophysics*. Vol. 5. Issue 7. pp. 4-20.

[69]. Al-Kushi A. G., Mohamed, E. E, Naser A. E, Osama, A. O., & Eslam, A. (2013). Pathological Comparative Studies on Aqueous and Ethanolic Extracts of *Zingiber officinale* on Antioxidants and Hypolipidemic Effects in Rats. *Life Science Journal*. Vol. 10. Issue 2. pp. 2393-2403

[70]. Ayala, Munoz, M, F., & Arguelles, S. (2014). Lipid Peroxidation Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Neonenal. *Oxidative Medicine and Cellular Longevity*. pp.1 – 31.

[71]. Skoczynska, A. (2008). Genetic aspects of hypertensive effect of lead. *Medical Proceduer*. vol 59, pp. 325–332.

[72]. Ahamed, M. & Siddiqui, M.K.J. (2007). Low level lead exposure and oxidative stress: Current opinions. *Clin. Chem. Acta*. vol. 383. pp. 57–64.

[73]. Kelada, S. N., Shelton, E., Kaufmann, R. B., & Khourg, M. J. (2001). δ -aminolevulinic acid dehydratase genotype and lead toxicity: a huge review. American Journal epidemiology. Vol. 154. pp. 1 – 13.

[74]. Gurer, H., & Ercal, N. (2000). Can antioxidants be beneficial in the treatment of lead poisoning? *Free Radicxal Biological Medicine*. Vol. 29: pp. 927 – 945.

[75]. Bechara, E. J. H. Oxidative stress in acute intermittent porphyria and lead poisoning may be triggered by 5-aminolevulinic acid. *Brazillian. J. Med. Biol. Res.* **29:**841–851; 1996.

[76]. Farant, J. P.; Wigfield, D. C. Biomonitoring lead exposure with ALAD activity ratios. *Int. Arch. Occup. Environ. Health* **51:**15– 24; 1982.

[77]. Hermes-Lima, M. (19995). How do Ca21 and 5-aminolevulinic acidderived oxyradicals promote injury to isolated mitochondria? *Free Radical Biological Medicine*. **19:**381–390.

[78]. Lawton, L. J.; and Donaldson, W. E. (1991). Lead-induced tissue fatty acid alterations and lipid peroxidation. *Biology Trace Element Research*. Vol. 28**. pp.** 83– 97.

[79]. Xhyrel J J, Nikko L G, & Marlon C. P. (2016) Relative antioxidant efficacy of α-tocopherol and ascorbic acid on blood lead, haemoglobin and haematocrit level of lead-exposed Rattus norvegicus (albino rat). Der Pharmacia Lettre Vol. 8. pp. 169-179.

[80]. 80. Halliwell, B. and Gutteridge, J. C. (1999): Free Radicals in Biology and Medicine, 3rd ed., London, England: Oxford Univ. Press.

[81]. Valko et al., 2006). Valko, M., Rhodes, C. J., Monocol, J., Izakovic, M. & Mazur, M. (2006). Free radicals, metals and antrioxidants in oxidative stress-induced cancer. *Journal Chemistry Biology Interact*. pp. 189:191.

[82]. Kalender, S., Uzun, F.G., Demir, F., Uzunhisarcikli, M., & Aslanturk, A. (2013). Mercuric chloride-induced testicular toxicity in rats and the protective role of sodium selenite and vitamin E. *Food Chemistry and Toxicology*. Vol. 55. pp. 456 – 462

[83]. Aslanturk, A., Uzunhisarcikli, M., Kalender, S., & Demir, F. (2014). sodium selenite and vitamin E in preventing mercuric chloride-induced renal toxicity in rats. *Food Chemistry Toxicology*. Vol. 70. pp. 185 - 190.

[84]. Perottoni, J., Rodrigues, O.E.D., Paixao, M.W., Zeni, G., Lobato, L.P., Braga, A.L., Rocha, J.B.T., & Emanuelli, T. (2004). Renal and hepatic ALA-D activity and selected oxidative stress parameter of rats exposed to inorganic mercury and organoselenium compounds. *Food Chem. Toxicology*. Vol. 42. pp. 17 – 28.

[85]. Ferbal Ö., Arzu Ö, Suna G., Mehmet B., & Serap Y. (2014). Effects of dietary selenium of organic form against lead toxicity on the antioxidant system in Cyprinus carpio**.** Fish Physiol Biochem. Vol. 40. Issue. 2. pp. 355-63**.**

[86]. Atroshi, F., Rizzo, A., Biese, I., Veijalainen, P., Salonecill, H., & Anderson, K. (1991). Fumonisin B1- Induced BNA damage in rat liver and spleen: effects of pretreatment with coenzyme. Q10, L-carnitine, alpha – tocopherol and selenium. *Pharmacological Research*. Vol. 40. pp. 459-467.

[87]. Aslam et al, 2010; Aslam, F., Khan, A., Khan, M. Z., Sharaf, S., Gul, S. T., & Sakemi, M. K. (2010). Toxico – Pathological changes induced by cypermethrin in broiler chicks: their attenvation with vitamin E and selenium. *Exp Toxicol pathology*. Vol. 62. pp. 441-450.

[88]. Schwenke, D. C., & Behr, S. R. (1998). Vitamin E combined with selenium inhibits atherosclerosis in hypercholestaemic rabbit independently of effects on Plasma cholesterol concentration. *Res*, Vol. 83. pp. 366-377.

[89]. Ebuehi, O.A.T., Ogedegbe, R.A., & Ebuehi, O.M. (2012). Oral Administration of Vitamin C and Vitamin E amelioratesLead-induced Hepatotoxicity and Oxidative Stress in the Rat Brain. *Nig. Qt J. Hosp. Med.* Vol. 22. Issue 2. Pp. 2-3.

Onitsha, Enebrayi N, et. al. "Influence of Vitamin E and Selenium on Reproductive Hormones and Lipid Peroxidation Levels in Lead-induced Toxicity in Female Wistar Rats.." *IOSR Journal of Environmental Science, Toxicology and Food Technology* (IOSR-JESTFT), 15(2), (2021): pp 01-09.