



CYTOPROTECTIVE EFFECT OF *LAWSONIA INERMIS* LEAVE EXTRACT IN ALUMINIUM- INDUCED OXIDATIVE STRESS ON SEX HORMONES PROFILE IN ADULT MALE WISTAR RAT.

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Article Received on 24/01/2017

Article Revised on 14/02/2017

Article Accepted on 06/03/2017

ABSTRACT

Henna (*Lawsonia inermis*) has been used cosmetically and mechanically for over 9,000 years. Metals can cause hormonal imbalance by affecting the neuroendocrine system, disrupting the secretion of androgens from Leydig cells. The aim of this study was to investigate: 'Cytoprotective effect of *Lawsonia inermis* Leaves Extract on Aluminium-induced Oxidative stress in the Hormone profile of Adult male Wistar rat'. The objectives were to study the antioxidant effect of *Lawsonia inermis* leaves extract on aluminum induced oxidative stress on the Hormone profiles: leutenizing hormone (LH), follicular stimulating hormone (FSH) and testosterone. Thirty five adult male Wistar rats were used, with an average weight of 100-196g. *Lawsonia inermis* extracts and aluminum chloride (AlCl₃) were administered for a period of three (3) weeks with five (5) rats per group. Group A (control): Given rat pellets and distilled water. Group B: Given 60mg/kg/d extract of aqueous *Lawsonia inermis*. Group C: Given 0.5mg/kg/d of AlCl₃. Group D: Given 0.5mg/kg/d of AlCl₃ and low dose 60mg/kg/d of aqueous *Lawsonia inermis* orally. Group E: Given 0.5mg/kg/d of AlCl₃ and medium dose 75mg/kg/d of aqueous *Lawsonia inermis* orally. Group F: Given 0.5mg/kg/d of AlCl₃ and high dose 100mg/kg/d of aqueous *Lawsonia inermis* orally. Group G: Given 0.5mg/kg/d of AlCl₃ and 5mg/Kg/d Ascorbic acid in distilled water orally. Twenty four hours after the last administration, the animal were weighed and thereafter sacrificed by the use of chloroform as a sedative. The organs were located, removed and weighed using an electronic sensitive analytical balance (Sartorius). Results, showed no significant changes in the weight of Pituitary gland and brain but there was significant changes statistically on the hormone profile. In conclusion, *Lawsonia Inermis* has been clearly shown to increase reproductive hormones significantly, rendering free radical harmless and markedly lessens stress deleterious effect.

KEYWORDS: Oxidative Stress, Sex hormones, *L. inermis*.

INTRODUCTION

The potential herbal medicines and medicinal plant research results in health care is no longer in doubt having gained recognition in several nations of the world and World Health Organization (WHO).^[33, 43]

Lawsonia inermis commonly known as henna belongs to the family Lythraceae and genus Lythrum. Henna a common name for a small shrub and for the dye that is obtained from its leaves. The shrub is also called alkanna mignonette tree, El-henna and Egyptian priest. Phytochemical screening of the henna leaf extracts showed the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids.^[35]

According to Jain^[18], the phytochemical investigation of henna leaf shows total ash (14.60%), acid insoluble ash (4.50%) and water soluble ash (3.0%). Alcoholic extract and aqueous extract carbohydrate, glycosides, tannins, phenolic compounds, gums and mucilage were present in good quantity and saponins, alkaloids, phytosterols, fixed oils, fats, proteins, amino acids, volatile oils were absent. There is evidence of the plant having wound healing properties.^[6]

Aluminum is the most abundant metal and the third most common element in the earth's crust.^[28] It is being found abundantly as trioxosilicate (IV) in the rocks and Clays. The impact of aluminum on human health has been

increasingly alarming in recent years.^[28] Aluminum, the most abundant metal, makes up about 8% of the Earth's crust and is found in combination with oxygen, silicon, fluorine and other elements in the soil, rocks, clay and gems.^[37]

The primary sources of aluminum are: corn, yellow cheese, salt, herbs, spices, tea, cosmetics, cookware, utensils and containers.^[9,34] It is included in food additives and toothpaste¹ and broadly used in medicines such as antacids, phosphate binders, buffered aspirin, vaccines and injectable allergens.^[10,24]

The American Association of Poison Control Centers reported 813 single exposures to aluminum in 2013, with seven moderate outcomes, no major outcomes and no deaths.^[8, 9] Advances in nanotechnology have led to the exposure of humans to engineered aluminum nanomaterials (NMs) that could potentially induce genomic changes.^[9,29]

The oral bioavailability of aluminum ion in man and experimental animals from drinking water has been estimated to be in the range of 0.3%, whereas the bioavailability of aluminium from food and beverages generally is considered to be lower, about 0.1%.^[29] The degree of water solubility of an aluminum compound appears to increase the bioavailability of the aluminium ion, the presence or absence in the intestines of dietary ligands may either increase (e.g. citrate, lactate and other organic carboxylic acid complexing agents, fluoride), or decrease the absorption (e.g. phosphate, silicon, polyphenols). After absorption, aluminium distributes to all tissues in animals and humans and accumulates in some, in particular bone.^[39] The main carrier of the aluminium ion in plasma is the iron binding protein, transferring about 95% bind to transferrin and albumin transvasicularly and eliminated renaly.^[27] Aluminium can enter the brain and reach the placenta and fetus.^[27]

The Agency for Toxic Substances and Disease Registry (ATSDR) reported that aluminum accumulates mainly in the bone, liver, testes, kidneys and brain.^[2] Patients on dialysis^[5] or undergoing long-term treatment with total parental nutrition^[23] tend to accumulate this metal in different organs. The toxicological effects on humans include encephalopathy^[5], bone disease^[22], anemia and skeletal system disease.^[36] Metals can cause hormonal imbalance by affecting the neuroendocrine system, disrupting the secretion of androgens from Leydig cells or Inhibin B from Sertoli cells.^[19]

Aluminum induces oxidative stress in animals; this is indicated by a sharp rise in lipid peroxidation potentials. This may be due to higher lipid content in the testis.^[28, 17] Investigations in humans and experimental animal models provide evidence for the reproductive toxicity of aluminum exposure in males. Suppressed testosterone production, sexual behavior and fertility were also observed in animals exposed to aluminum.^[44]

Infertility is a worldwide problem, affecting 8-15% of the couples in their reproductive age. However, the incidence rate of infertility varies across the globe and Nigeria falls within the infertility belt of Africa.^[3] Abnormal hormone production has been noted as a male causative factor of infertility.^[4]

Hormone regulation of male reproductive function is regulated by follicle stimulating hormone (FSH), which in males stimulates the spermatogenic epithelium, and luteinizing-hormone (LH), which in males stimulates testosterone production by Leydig cells in the interstitial tissue. Follicle-stimulating hormone (FSH) is a hormone found in humans and other animals. It is synthesized and secreted by gonadotrophs of the anterior pituitary gland. FSH regulates the development, growth, pubertal maturation and reproductive processes of the body. FSH and Luteinizing hormone (LH) act synergistically in reproduction.

Testosterone is a steroid hormone from the androgen group and is found in mammals, reptiles, birds and other vertebrates.^[11] In mammals, testosterone is primarily secreted in the testes of males and the ovaries of females, although small amounts are also secreted by the adrenal glands. It is the principal male sex hormone and an anabolic steroid. In males, testosterone is primarily synthesized in Leydig cells. There are few studies to suggest that testosterone together with FSH promotes spermiogenesis by promoting adhesion of round spermatids to Sertoli cells.^[31]

Testosterone regulates its own secretion by negative feedback mechanism. It acts on hypothalamus and inhibits the secretion of luteinizing hormone-releasing hormone (LHRH). When LHRH secretion is inhibited, LH is not released from anterior pituitary, resulting in the stoppage of testosterone secretion from testes. On the other hand, when testosterone production is low, lack of inhibition of hypothalamus leads to secretion of testosterone through LHRH and LH.

Luteinizing hormone (LH, also known as lutropin) is a hormone produced by the anterior pituitary gland. In *males*, where LH had also been called interstitial cell-stimulating hormone (ICSH), it stimulates Leydig cell production of testosterone.^[25] In both males and females, LH is essential for reproduction. In the male, LH acts upon the Leydig cells of the testis and is responsible for the production of testosterone, an androgen that exerts both endocrine activity and intra-testicular activity on spermatogenesis.^[30]

This study is aimed at investigating: 'Cytoprotective effect of Lawsonia *inermis* Leaves Extract on Aluminium-induced Oxidative stress in the Sex hormone profile of Adult Wistar rat'. The objectives are to study the antioxidant effect of Lawsonia *inermis*leave extract on aluminum induced oxidative stress on the Hormone

profiles: leutenizing hormone (LH), follicular stimulating hormone (FSH) and testosterone.

MATERIALS AND METHODS

Aluminium Chloride and Normal saline were bought in Mich-Deson Hospital Equipment store, Upper Taiwo, Ilorin. The ELIZA kits for hormone profiles were bought from Nums Diagnostic Centre, Suleja.

Preparation of Extracts

The plant was obtained from selected natural environment was professionally identified. The plant leaves were washed with water, cut into pieces, dried in a cool environment. The dried plant leaves were pulverized into coarse powder in a grinding machine. The filtrate was concentrated using Rotary evaporator (Buchi) and further concentrated to dryness at 50°C in an electric oven (GallenKamp). After drying it was stored in the refrigerator at 4°C until needed for use.

Acute Toxicity Test (Ld50)

Fifteen mice were used to conduct the above test to determine the safe dosages and lethal dosage levels, according to Holford.^[46] They were grouped into five (5), with three (3) mice per group. The acute toxicity of the Hydro-Methanol Extract of *Lawsonia Inermis* extract was assessed by LD50 calculation, using a limit dose test at a limit dose of 1000mg/kg bw of the extract after oral administration in mice (three animals per group) (OECD-OCDE 425 Guide). Using the oral route, the animals showed dose-dependent signs of toxicity, ranging from lack of appetite, depression, immobility and respiratory distress to death. LD50 for *Lawsonia Inermis* extract is 0.75g while the safe dose is 0.1g/Kg b.w.

Determination of the Dosage of the Extract to Administer

The choice of dosage based on the acute toxicity test (Ld50) above, the safe dose of *Lawsonia inermis* is 0.1g/Kg or 100mg/Kg body weight. The highest dose is 100mg/Kg, medium dose is 75mg/Kg and the lowest dose is 60mg/Kg.

Breeding of the Animals

Thirty five adult male Wistar rats and fifteen mice were used, with an average weight of 100-196g. The rats, after procurement, were housed in cages (made from wood, wire gauze and net) under natural light and dark cycles at room temperature in the animal house of the Faculty of Basic Medical Sciences, University of Ilorin. The floor of the cages were made with wood to make it comfortable for the rats and it was covered with sawdust to provide a soft floor for the rats and to make cleaning of the cage convenient when littered. They were fed with rat pellets purchased from approved stores by University of Ilorin and water was given *ad libitum*. They were grouped and left to acclimatize for 2 weeks before the study commences.

Grouping

The total numbers of animals were thirty five. They were grouped into one (1) control and six (6) experimental groups with consideration towards size variation. Using a feeding tube (size-6), distilled water and *Lawsonia inermis* extracts were administered to the control and treated animals respectively for a period of three (3) weeks.

Group 1 (control): (n = 5): Given rat pellets and distilled water.

Group 2: (n = 5): Given 60mg/kg/d extract of *Lawsonia inermis* and pellets.

Group 3: (n = 5): Given 0.5mg/kg/d of aluminum chloride in distilled water and pellets.

Group 4: (n = 5): Given 0.5mg/kg/d of aluminum chloride and low dose 60mg/kg/d of *Lawsonia inermis* in distilled water orally.

Group 5: (n = 5): Given 0.5mg/kg/d of aluminum chloride and medium dose 75mg/kg/d of *Lawsonia inermis* orally.

Group 6: (n = 5): Given 0.5mg/kg/d of aluminum chloride and high dose 100mg/kg/d of *Lawsonia inermis* in distilled water orally.

Group 7: (n=5): Given 0.5mg/kg/d of aluminum chloride and 5mg/Kg/d Ascorbic acid in distilled water orally.

Animal Sacrifice and Sample collection

Twenty four hours after the last administration, the animal were weighed and thereafter sacrificed by the use of chloroform as a sedative. Immediately animals were euthanized the blood samples were collected using syringes by cardiac puncture through the mid-clavicular line (i.e. apex beat of animals heart was located) into EDTA bottle.

Serum Assay Testosterone Procedure^[42]

The blood will be collected by ventricular puncture into plain redtop venipuncture tubes. Blood samples will be spun at 3000 revolution per minute for 10 minutes in an angle head centrifuge at 25°C and serum testosterone will be assayed. The samples will be assayed in batches using the Ezyme linked immunoassay (ELIZA) method. The microwell kit will be from Biotec laboratories Ltd, UK. With ten micron (10µl) of the standard, the specimens and control will be dispensed into the number of coated wells to be used. Hundred micron (100 µl) testosterone conjugate reagent will be added and then fifty micron (50 µl) of anti-testosterone reagent. The contents of the microwell will be thoroughly mixed and then incubated for 20 minutes at room temperature. The reaction will be stopped with 100µl of 1M hydrochloric acid. Absorbance will be measured with an automatic spectrophotometer (Rayto: RT-2100C, Microplate Reader) at 450nm.

Luteinizing Assay Procedure^[41]

LH was quantitatively determined according to manufacturer instruction based on the method of Wennink.^[41] Essentially, biotinylated monoclonal and enzyme labeled antibodies are directed against LH

epitope—the immunologically active site. The reaction between LH antibodies and native LH forms a sandwich complex that binds with the streptavidin coated to the well. Following the completion of the required incubation period, the enzyme-LH antibody bound conjugate is separated from unbound enzyme-LH conjugated by separation and decantation. The activity in the antibody-bound fraction is directly proportional to the native antigen concentration. The activity of the enzyme present on the surface of the well was quantified by reaction with suitable substrate to produce color.

Twenty five microns (25 μ l) of the standard, the specimens and controls will be dispensed into appropriate wells. Twenty five microns (25 μ l) of enzyme conjugate reagent will be pipetted into the wells and thoroughly mixed for 30seconds and incubate at a temperature of 36°C for 60minutes. The microtiter wells will be rinsed and flicked 5 times with three hundred micron (300 μ l) of washing solution. The wells will be struck sharply with absorbent paper to remove all residual all residual water. Hundred micron (100 μ l) of TMB substrate solution was added to each well and mixed and then incubate at room temperature for 15minutes. The reaction was stopped using hundred micron (100 μ l) of stopping solution. The samples will be gently mixed for 30 seconds until the blue colour changes to yellow. Absorbance will be read at 450nm

with Rayto: RT-2100C, Microplate Reader within 15 minutes.

Follicle Stimulating Hormone Assay Procedure^[38]

25microns (μ l) of the standard, the specimens and controls will be dispensed into appropriate wells. 50 μ l of enzyme conjugate reagent will be pipetted into the wells and thoroughly mixed for 30seconds and incubate at a temperature of (36°C) for 60minutes. The microtiter wells will be rinsed and flicked 5 times with three hundred micron (300 μ l) of washing solution. The wells will be struck sharply with absorbent paper to remove all residual water. Hundred micron (100 μ l) of TMB substrate solution will be added to each well and mixed and then incubate at room temperature for 15minutes. The reaction will be stopped using hundred micron (100 μ l) of stopping solution. The samples will be gently mixed for 30 seconds until the blue colour changed to yellow. Absorbance will be read at 450nm with a microtiter well reader within 15 minutes.

Statistical Analysis

All biochemical results were expressed as Mean \pm SD significant differences among the groups were determined by one-way analysis of variance (ANOVA) followed by Tukey Scheffe LSD Alpa post-test or unpaired Student's t-test using the SPSS statistical analysis program. Statistical significance was considered at $p < 0.05$.

RESULT

Table 1: Mean and standard error of mean (sem) for Pituitary gland and Brain weights

GROUPS	Pituitary Weight (mg) \pm sem	Brain weight (g) \pm sem
GRP 1	.01 \pm 0.003	1.6 \pm 0.03
GRP 2	0.35 \pm 0.36	1.6 \pm 0.08
GRP 3	0.42 \pm 0.42	1.7 \pm 0.18
GRP 4	0.49 \pm 0.48	1.6 \pm 0.09
GRP 5	0.15 \pm 0.14	1.3 \pm 0.19
GRP 6	0.58 \pm 0.55	1.6 \pm 0.12
GRP 7	0.01 \pm 0.001	1.5 \pm .06

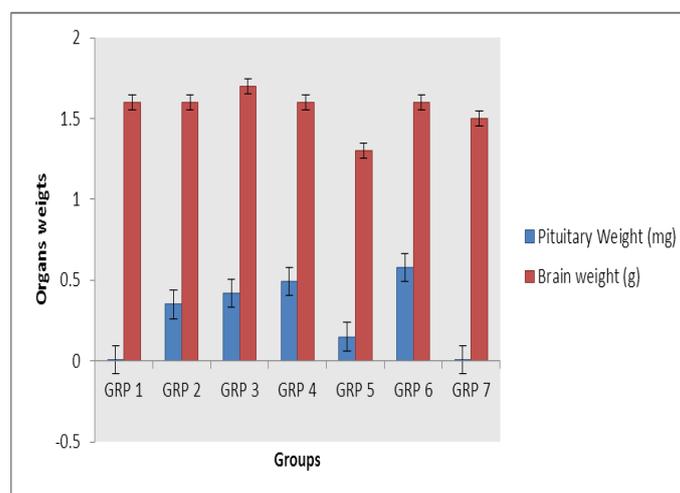


Figure 1: Variation in weights of Pituitary gland and Brain.

There were no statistically significant changes in the Pituitary gland weight difference of the experimental

animals in all the groups ($p > 0.05$). Group 6 (100mg/kg/d of *Lawsonia inermis* with AIC13) had the highest value

and groups 1 and 7 had the lowest value (Table 1 & Figure 1).

Table 2: The Distribution of Mean and standard error of mean (sem) for Malondialdehyde enzyme.

GROUPS	MDA ($\mu\text{mol/L}$)
GRP 1	1.9 ± 0.18
GRP 2	2.5 ± 0.03
GRP 3	4.6 ± 0.13
GRP 4	4.0 ± 0.09
GRP 5	3.6 ± 0.09
GRP 6	3.3 ± 0.03
GRP 7	2.9 ± 0.06

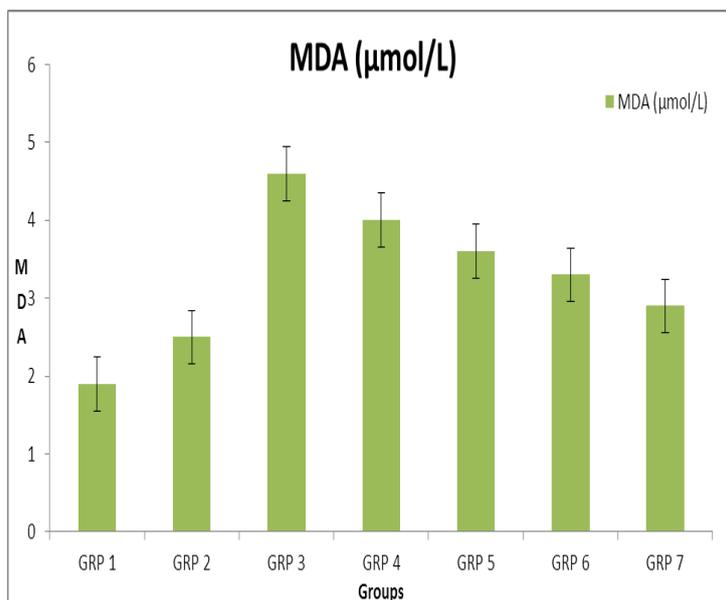


Figure 2: Variation in the Malondialdehyde enzyme

Statistically, the biochemical enzyme: Malondialdehyde were markedly significant ($p < 0.05$). Group 3 (Given aluminum chloride alone) has the highest value $4.6 \pm$

0.13 (IU/L), while group 1 (control) has the lowest value 1.9 ± 0.18 (IU/L) (Table 2 & Figure 2).

Table 3: The Distribution of Mean and standard error of mean (sem) for the Hormone Profile

GROUPS	FSH(MIU/ml)	LH(MIU/ml)	Testosterone(ng/ml)
GRP 1	0.7 ± 0.06	0.6 ± 0.02	6.8 ± 0.05
GRP 2	1.2 ± 0.42	1.2 ± 0.35	7.0 ± 0.05
GRP 3	0.3 ± 0.07	0.2 ± 0.03	5.3 ± 0.16
GRP 4	0.5 ± 0.03	0.4 ± 0.02	6.0 ± 0.02
GRP 5	0.5 ± 0.02	0.5 ± 0.01	6.2 ± 0.04
GRP 6	0.6 ± 0.03	0.5 ± 0.002	6.4 ± 0.07
GRP 7	0.6 ± 0.07	0.6 ± 0.01	6.7 ± 0.03

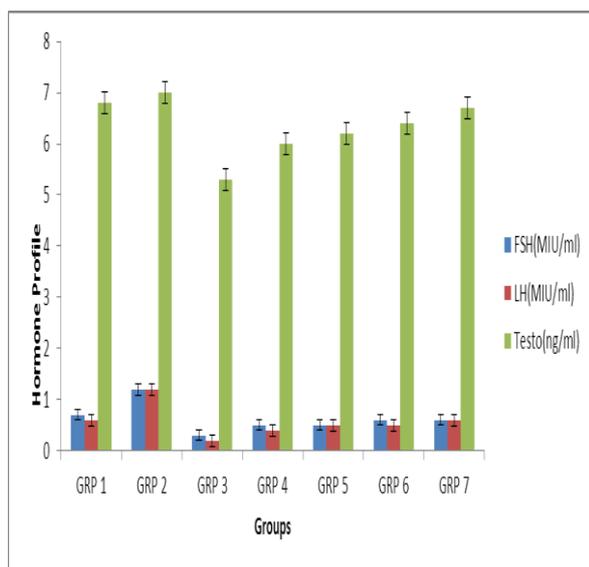


Figure 3A: Variation in the Hormone Profile

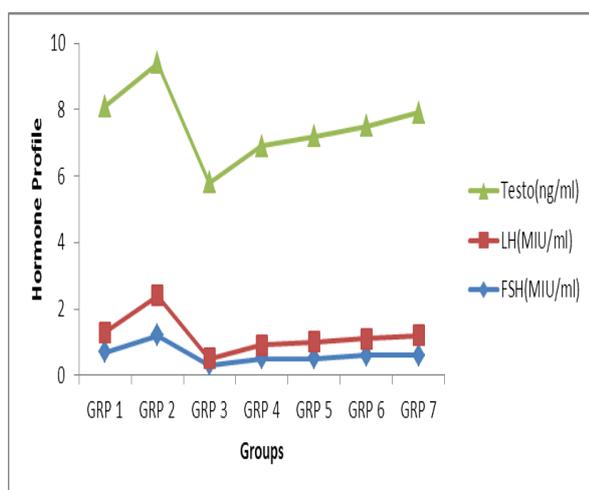


Figure 3B: Variation in the Hormone Profile

Statistically, the hormone profiles were markedly significant ($p < 0.05$), with mean and standard error of mean for FSH (MIU/ml) for groups (1-7): (0.7 ± 0.06 , 1.2 ± 0.42 , 0.3 ± 0.07 , 0.5 ± 0.03 , 0.5 ± 0.02 , 0.6 ± 0.03 and 0.6 ± 0.07) while group 2 with *Lawsonia inermis* extract having the highest value (Table 3, Figure 3A, B). LH (MIU/ml) for groups (1-7): (0.6 ± 0.02 , 1.2 ± 0.35 , 0.2 ± 0.03 , 0.4 ± 0.02 , 0.5 ± 0.01 , 0.5 ± 0.002 , 0.6 ± 0.01) also group 2 with *Lawsonia inermis* extract having the highest value (Table 3, Figure 3A, B). Testosterone (ng/ml) for groups (1-7): (6.8 ± 0.05 , 7.0 ± 0.05 , 5.3 ± 0.16 , 6.0 ± 0.02 , 6.2 ± 0.04 , 6.4 ± 0.07 , 6.7 ± 0.03) while group 2 with *Lawsonia inermis* extract having the highest value (Table 3, Figure 3A, B). Figure 3B, shows that the hormones follow the same pattern, group 2 that were given *Lawsonia inermis* leaves extract alone were the highest followed by group 1 (Control), group 3 (aluminum alone) was the least while groups 4-6 were given concomitantly aluminum chloride and low, medium and high doses *Lawsonia inermis* leaves extract which increase based on the doses i.e they are dose dependent; while group 7 was given aluminum chloride

and ascorbic acid this was higher than the highest dose of *Lawsonia inermis* leaves extract.

DISCUSSION

The findings revealed no statistically significant difference on the weights of both the Pituitary gland and brain, with the animal weight ($p > 0.05$) (Tables 1) (Figures 1). Considering Tables 1; Figures 1, group 6 that received high dose aqueous *Lawsonia inermis* leaf extract and AlCl₃ had the highest pituitary gland weight while group 1 (control) and group 7 had the lowest weight. Also, group 3 that received AlCl₃ alone had the highest brain weight while group 5 which received medium dose had the lowest brain weight. From this finding, weight did not follow the normal trend and cannot be used in this study to explain significant changes.

The biochemical findings shows that malondialdehyde were markedly significant ($p < 0.05$). Group 3 that was given aluminum chloride alone has the highest value while group 1 (control) has the lowest value according to Table 2. Exposure to Aluminum chloride induced oxidative stress and leads to increase value of malondialdehyde in group 3 while group 6 with high dose of aqueous *Lawsonia inermis* leaf extract also recorded low value of malondialdehyde. This finding is actually in agreement with some of these authors.

According to Darley-Usman^[13] production of malondialdehyde (MDA) is an end product of Lipid peroxidation. Oxidative stress is produced by the peroxidation and oxidation of many cell lipids; proteins, carbohydrates, and nucleic acids.^[26] Exposure to high concentrations of certain metals has also been shown to cause oxidative stress and high metal doses increase oxidative damage and deplete antioxidants in the testes of rats.^[26,40] Testicular oxidative stress plays a role in a number of conditions known to be detrimental to male fertility (sex hormones). Oxidative stress in any tissue results from an imbalance between the production of reactive oxygen species (ROS) and their efficient removal by available antioxidant systems. Reactive oxygen species are small, oxygen-based molecules that are highly reactive because of unpaired electrons.^[32] Moreso, this study reveals that during administration of the aqueous *Lawsonia inermis* leaf extract, there were statistically significant ($p < 0.05$) effect on the reproductive hormone profiles of an adult Wistar rats. The effect of aqueous *Lawsonia inermis* leaf extract in enhancing male fertility clearly manifested in the treated groups compared with the control (Table 3). The results illustrated that the *Lawsonia inermis* leaf extract significantly increased the level of Luteinizing hormone (LH), follicle-stimulating hormone (FSH) and Testosterone. This could provide an interpretation for the finding in this study why the *Lawsonia inermis* leaf extracts showed an enhanced hormonal level. Regulation of the reproductive system is a process that requires the action of hormones from the pituitary gland, the adrenal

cortex and the gonads. During puberty, in both males and females, the hypothalamus produces gonadotropin-releasing hormone (GnRH), which stimulates the production and release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary gland. These hormones regulate the gonads (testes in males and ovaries in females); they are called gonadotropins. In both males and females, FSH stimulates gamete production and LH stimulates production of hormones by the gonads. An increase in gonad hormone levels inhibits GnRH production through a negative feedback loop^[45].

It was clearly shown based on this study, group 3 (AlCl₃ treated group) mean and standard error of mean were significantly reduced (Table 3, Figures 3A, B). This is similar to Jensen^[19], findings that metals (Inorganic Lead) may affect the male reproductive system directly, when they target specific reproductive organs, or indirectly, when they act on the neuroendocrine system and it can cause hormonal imbalance by affecting the neuroendocrine system, disrupting the secretion of androgens from Leydig cells or Inhibin B from Sertoli cells. Similarly^[7], equally said metals can affect the testis size, semen quality, the secretory function of the prostate and seminal vesicles, the reproductive endocrine function and can lead to the loss of fertility and libido or to impotence.

One of the major mechanisms behind metal toxicity has been attributed to oxidative stress. Oxidative stress in any tissue results from an imbalance between the production of reactive oxygen species (ROS) and their efficient removal by available antioxidant systems. Papa and Skulachev^[32] reported that ROS are small, oxygen-based molecules that are highly reactive because of unpaired electrons. The most prominent ROS are the superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂) and the hydroxyl ion (OH[•]). Testicular oxidative stress plays a role in a number of conditions known to be detrimental to male fertility. According to Karlsson^[20]; Dandekar^[12], lipid peroxidation of sperm membrane is considered to be the key mechanism of ROS-induced sperm damage leading to infertility.

Concomitantly, aluminum chloride and low, medium and high doses aqueous *Lawsonia inermis* leaves extract were given to groups 4-6, rats administered with the high and medium doses of *Lawsonia inermis* leaf extract are reproductively increase to those that were given low doses that is they are dose dependent (Table 3). Concomitantly, aluminum chloride and varying dosages of *Lawsonia inermis* leaf extract, significantly lessens stress deleterious effect on sex hormone, not by blunting stress reactivity but by significantly limiting testicular lipid peroxidation. These results may be attributing to presence of flavonoids that can ameliorate oxidative stress-related testicular impairments in animal tissues.

Lastly, concomitant administration of aluminum chloride and ascorbic acid markedly reduced the testicular lipid peroxidation (Table 3). According to Kehrer, and Smith^[21] ascorbic acid (antioxidants) are constantly rendering free radicals harmless. They protect the cells and organ systems of the body against reactive oxygen species. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals.

CONCLUSION

Lawsonia inermis leaf extract has been clearly shown to be a phenolic compound and a good antioxidant agent against oxidative stress induced by heavy metal toxicity. It has also increase reproductive hormones significantly, rendering free radical harmless and markedly lessens stress deleterious effect on sex hormones.

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