



**EFFECT OF *LAWSONIA INERMIS* LEAVE EXTRACT IN ALUMINIUM- INDUCED  
OXIDATIVE STRESS ON BIOCHEMICAL PARAMETERS OF ADULT MALE WISTAR  
RAT.**

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**ABSTRACT**

Henna (*Lawsonia inermis*) is a perennial shrub, that is mildly anti-inflammatory and is effective against ringworm and other fungal diseases. All isolated compounds exhibited antioxidant activity. Toxic effects of aluminum poisoning can cause asthenospermia, hypospermia, teratospermia and reduction in sperm count. The aim of this study was to investigate: 'Effect of *Lawsonia inermis* Leaves Extract on Aluminium-induced Oxidative stress on biochemical parameters of Adult male Wistar rat'. The objectives were to study the antioxidant effect of *Lawsonia inermis* aqueous leaves extract on aluminum induced oxidative stress on the Biochemical parameters: Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Malondialdehyde (MDA). Thirty five adult male Wistar rats were used, with an average weight of 100-196g. *Lawsonia inermis* extracts and aluminum chloride (AlCl<sub>3</sub>) 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 AlCl<sub>3</sub>. Group D: Given 0.5mg/kg/d of AlCl<sub>3</sub> and low dose 60mg/kg/d of aqueous *Lawsonia inermis* orally. Group E: Given 0.5mg/kg/d of AlCl<sub>3</sub> and medium dose 75mg/kg/d of aqueous *Lawsonia inermis* orally. Group F: Given 0.5mg/kg/d of AlCl<sub>3</sub> and high dose 100mg/kg/d of aqueous *Lawsonia inermis* orally. Group G: Given 0.5mg/kg/d of AlCl<sub>3</sub> 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, biochemical findings shows that Superoxide dismutase and Glutathione Reductase were markedly significant (p<0.05). In conclusion, *Lawsonia inermis* aqueous leaf extract protects the cells and organ systems of the body against reactive oxygen species harmless, a good antioxidant agent against oxidative stress induced by heavy metal toxicity.

**KEYWORDS:** Asthenospermia, Hypospermia, *L. inermis*, Peroxidation, Teratospermia.

**INTRODUCTION**

Traditional medicines are becoming popular, due to high toxicity, adverse effects of orthodox medicaments and economy recession in this part of the world. This has led to sudden increase in the number of herbal industries in the drug market. Several plant species are used by various indigenous systems such as Siddha, Ayurveda, Unani for the treatment of different ailments.<sup>[5, 6, 7, 30, 36, 37]</sup>

Henna (*Lawsonia inermis*) a perennial shrub native of northern Africa, Asia and Australia is naturalized and cultivated in the tropics of America, Egypt, India and parts of the Middle East.

These findings depicted that all isolated compounds exhibited antioxidant activity comparable to that of ascorbic acid (2.5 mM).<sup>[26]</sup> Lawsone may be mildly anti-inflammatory and is effective against ringworm and other fungal diseases.<sup>[34]</sup>

Lawsone (2-hydroxy-1, 4 naphthoquinone) is the main ingredient of *L. inermis*. On oxidation of 100 μM phenanthridine by guinea pigs aldehyde oxidase, superoxide anion and hydrogen peroxide formation was found to be 6-10 % and 85-90 % respectively. Furthermore, the dyeing property of henna depends mainly on its principal colouring matter known as lawsone, 2- hydroxy-1:4 naphthaquinone (C<sub>10</sub>H<sub>6</sub>O<sub>3</sub>,

m.p.190° decomp.), the yield of which varies from 1% to 20%.<sup>[29]</sup>

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.<sup>[35]</sup>

The primary sources of aluminum are: corn, yellow cheese, salt, herbs, spices, tea, cosmetics, cookware, utensils and container.<sup>[2,32]</sup> It is included in food additives and toothpaste<sup>[1]</sup> and broadly used in medicines such as antacids, phosphate binders, buffered aspirin, vaccines and injectable allergens.<sup>[3, 22]</sup>

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%.<sup>[38]</sup> The main carrier of the aluminium ion in plasma is the iron binding protein, transferrin.<sup>[24]</sup> Aluminium can enter the brain and reach the placenta and fetus.<sup>[14]</sup>

Aluminum elicits toxic pathological changes in the testes, leading to atrophy of the organ.<sup>[25]</sup> Toxic effects of aluminum poisoning can cause asthenospermia, hypospermia, teratospermia and reduction in sperm count.<sup>[4]</sup> Aluminum has direct effect on the male gonads, consequently, the Aluminum factory workers experience hypofertility.<sup>[16]</sup> 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.<sup>[25, 28]</sup>

Aluminum exposure results in decreases in glutathione (GSH) levels which cause an increase in reactive oxygen species like hydrogen peroxide, hydroxyl radicals and superoxide radicals and superoxide radical ions, leading to increase lipid peroxidation, changes intercellular stability and damages detoxification.<sup>[28]</sup> It binds with heavy metals and the resulting water soluble metal is filtered out of the body.<sup>[19]</sup> Glutathione also directly reacts with free radicals and as substrate or cofactor, in enzymatic antioxidant reactions, may also protect the cell against oxidative stress.<sup>[13]</sup> Superoxide dismutase [SOD] may directly act as antioxidant enzymes involved in the inhibition of sperm lipid peroxidation [LPO]. A high reduced glutathione [GSH]/ oxidized glutathione [GSSG] ratio will help spermatozoa to combat oxidative insult.<sup>[18]</sup> It seems that the role of these biological antioxidants and their associated mechanisms is important in the treatment of infertility.

This study is aimed at investigating: 'Effect of *Lawsonia inermis* Aqueous Leaves Extract on Aluminium-induced Oxidative stress on Biochemical parameters of Adult Wistar rat'. The objectives are to study the antioxidant effect of *Lawsonia inermis* aqueous leave extract on aluminum induced oxidative stress on the Biochemical

parameters: Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Malondialdehyde (MDA).

## MATERIALS AND METHODS

Aluminium Chloride and Normal saline were bought in Mich-Deson Hospital Equipment store, Upper Taiwo, Ilorin. The Biochemical reagents 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 (1998). 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.

### Determination of Superoxide Dismutase (SOD) Activity<sup>[27]</sup>

Superoxide Dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480nm as described by Mishra and Fridovich, (1972). The reaction mixture (3ml) contained 2.95ml, 0.05M sodium carbonate buffer initiate the reaction. The reference cuvette contained 2.95ml buffer, 0.03ml of substrate (epinephrine) and 0.02ml of water. Enzyme activity was calculated by measuring the change in absorbance will be read by Chemistry Analyzer machine (MISPA Excel) at 480nm for 5 minutes.

### Determination of Glutathione Reductase<sup>[33]</sup>

The reduced glutathione (GR) content of testis tissue as non-protein sulphhydryls will be estimated according to the method described by Sedlak and Lindsay.<sup>[33]</sup> To the serum HCl reagent (thiobarbituric acid 0.37%, 0.24N HCl and 15% TCA) tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent is added. The absorbance is read at 532 nm against a blank. MDA will be calculated using the molar extinction coefficient for MDATBA-complex of  $1.56 \times 10^5 \text{ M}^{-1} \text{ CM}^{-1}$ . It can be read Directly from Chemistry Analyzer machine (Mispa Excel).

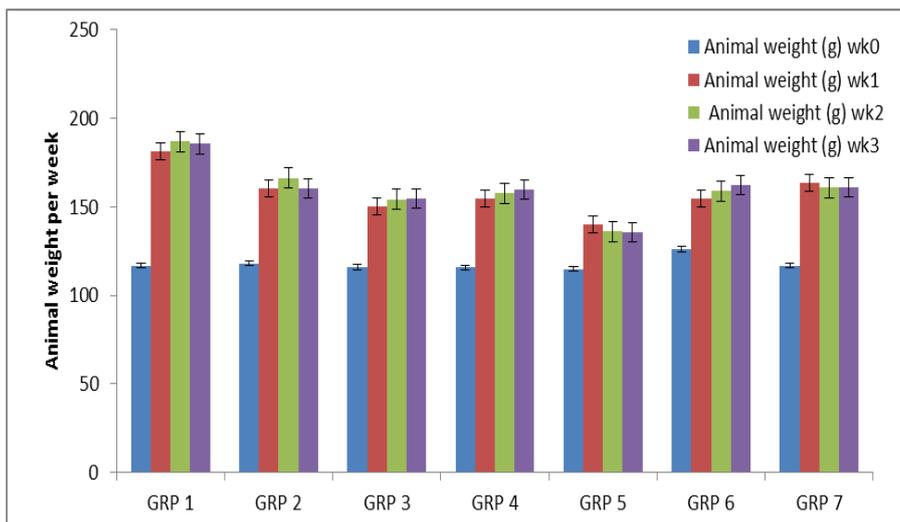
### Determination of Plasma Malondialdehyde

Malondialdehyde levels in plasma were measured according to the protocol outlined by Stocks and Domandy<sup>[41]</sup>. The reaction mixture contained 100µL of Plasma, 20% Trichloroacetic acid (1.0ml). The above were mixed and centrifuge at 2000rpm for 5mins to obtain the supernatant. 0.5ml of supernatant is mixed with 0.7% Thiobarbituric acid (1.0ml), the tubes were heated in a water bath at 100°C for 20 minutes and all tubes were cooled in water. The spectrophotometer was blanked using the reagent blank at 532nm. Absorbance of tests and standards were read and plasma Malondialdehyde level was calculated.

## RESULT

**Table 1: The Distribution of Mean and standard error of mean (sem) for the Animal Weights.**

GROUPS	Animal weight (g) wk0	Animal weight (g) wk1	Animal weight (g) wk2	Animal weight (g) wk3
GRP 1	116.9 ± 5.7	181.2 ± 5.0	186.8 ± 4.9	185.6 ± 4.0
GRP 2	118.1 ± 4.2	160.5 ± 4.2	166.4 ± 5.4	160.6 ± 4.7
GRP 3	115.9 ± 7.1	150.2 ± 7.2	154.2 ± 8.0	154.8 ± 9.2
GRP 4	115.7 ± 11.9	154.7 ± 7.0	157.8 ± 8.0	159.7 ± 7.8
GRP 5	115.0 ± 5.4	139.9 ± 9.2	136.0 ± 10.6	135.7 ± 15.4
GRP 6	126.1 ± 3.6	154.8 ± 7.4	159.0 ± 8.7	162.4 ± 9.6
GRP 7	116.8 ± 7.1	163.4 ± 9.6	161.0 ± 10.8	161.2 ± 9.7



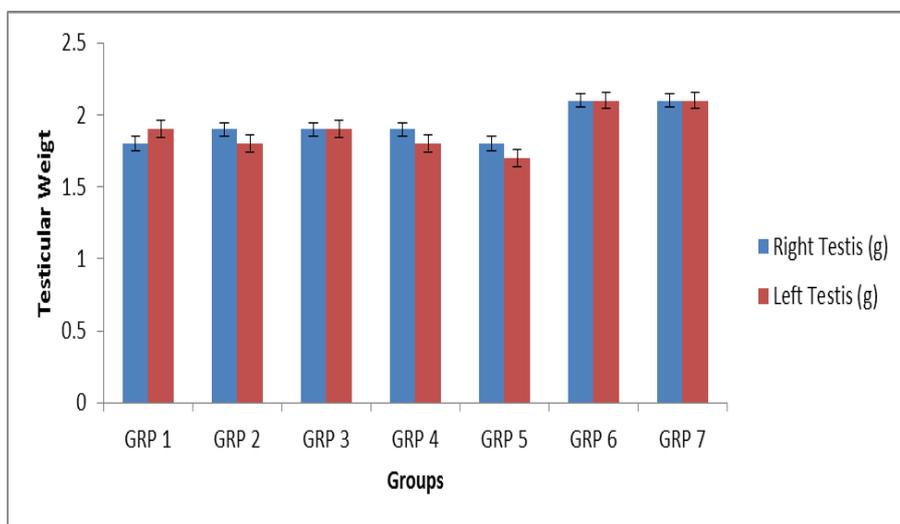
**Figure 1: Weekly Variation in Animal weights.**

There were no statistically significant changes in the animal weights difference of the experimental animals in all the groups in week zero and the third weeks ( $p > 0.05$ ) but there were statistically significant changes in the animal weights of the experimental animals in all the groups in first and second weeks ( $p < 0.05$ ). Table 1, shows that the mean and standard error of mean (sem) for the animal weights in the experimental animals in all the groups in first and second weeks were greatly increased as compared to week zero that is, week zero animal weight ( $116.9 \pm 5.7$ ,  $118.1 \pm 4.2$ ,  $115.9 \pm 7.1$ ,  $115.7 \pm 11.9$ ,  $115.0 \pm 5.4$ ,  $126.1 \pm 3.6$ ,  $116.8 \pm 7.1$ ) (g) while first week animal weights ( $181.2 \pm 5.0$ ,  $160.5 \pm 4.2$ ,  $150.2 \pm 7.2$ ,  $154.7 \pm 7.0$ ,  $139.9 \pm 9.2$ ,  $154.8 \pm 7.4$ ,  $163.4 \pm 9.6$ ) (g), second week animal weight ( $186.8 \pm 4.9$ ,  $166.4 \pm 5.4$ ,  $154.2 \pm 8.0$ ,  $157.8 \pm 8.0$ ,  $136.0 \pm 10.6$ ,  $159.0 \pm 8.7$ ,  $161.0 \pm 10.8$ ) (g); there were mild weights changes between second week and third week animal weight across the groups. Third week animal weights ( $185.6 \pm 4.0$ ,  $160.6 \pm 4.7$ ,  $154.8 \pm 9.2$ ,  $159.7 \pm 7.8$ ,  $135.7 \pm 15.4$ ,  $162.4 \pm 9.6$ ,  $161.2 \pm 9.7$ ) (g). More so, table 1, above shows that, group 1 (control) animal weights

increased drastically starting from the first week to the third week of treatment ( $116.9 \pm 5.7$ ,  $181.2 \pm 5.0$ ,  $186.8 \pm 4.9$ ,  $185.6 \pm 4.0$ ) (g) while group 5 (given medium dose *lawsonia inermis* with aluminum chloride) was greatly reduced throughout the treatment weeks ( $115.0 \pm 5.4$ ,  $139.9 \pm 9.2$ ,  $136.0 \pm 10.6$ ,  $135.7 \pm 15.4$ ) (g) followed by group 3 (given aluminum chloride alone) ( $115.9 \pm 7.1$ ,  $150.2 \pm 7.2$ ,  $154.2 \pm 8.0$ ,  $154.8 \pm 9.2$ ) (g).

**Table 2: The Distribution of Mean and standard error of mean (sem) for the Testicular Weights.**

GROUPS	Right Testis (g) ± sem	Left Testis (g) ± sem
GRP 1	1.8 ± 0.13	1.9 ± 0.13
GRP 2	1.9 ± 0.05	1.8 ± 0.09
GRP 3	1.9 ± 0.11	1.9 ± 0.14
GRP 4	1.9 ± 0.14	1.8 ± 0.10
GRP 5	1.8 ± 0.30	1.7 ± 0.32
GRP 6	2.1 ± 0.12	2.1 ± 0.16
GRP 7	2.1 ± 0.18	2.1 ± 0.21



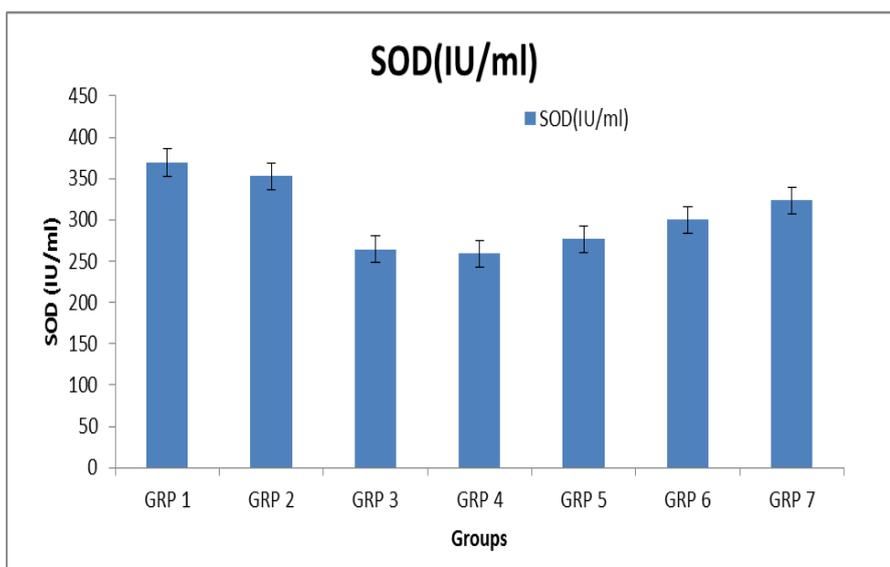
**Figure 2: Variation in Right and Left Testicular weight.**

There were no statistically significant changes in the testis weight difference of the experimental animals in all the groups ( $p > 0.05$ ). There were no much great difference between the mean weights across the groups. Table 2, shows that group 6 right and left testis with high dose of extract treatment and  $AlCl_3$  ( $2.1 \pm 0.12$ ,  $2.1 \pm 0.16$ ); group 7 right and left testis with Ascorbic acid and

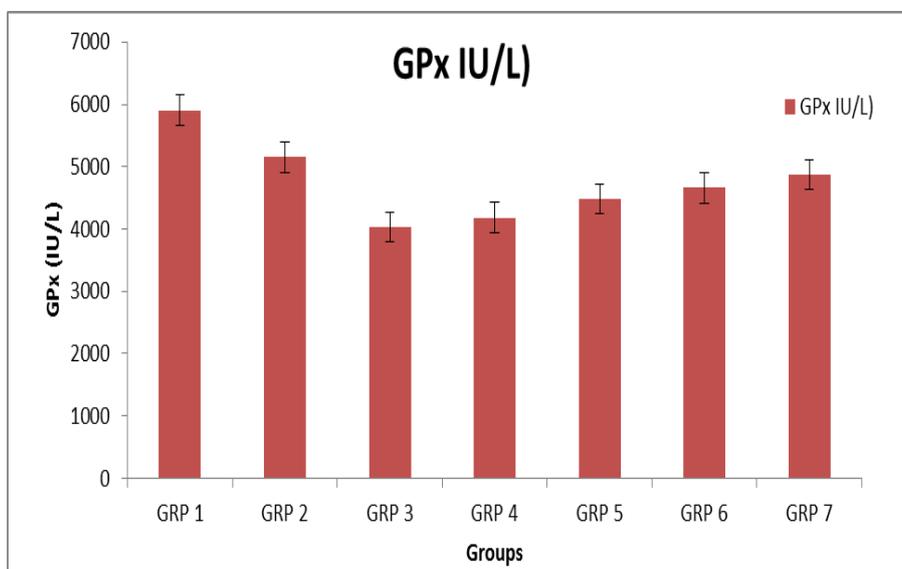
$AlCl_3$  ( $2.1 \pm 0.18$ ,  $2.1 \pm 0.21$ ) have the highest weights but group 5 right and left testis, with medium dose of extract and  $AlCl_3$  ( $1.8 \pm 0.30$ ,  $1.7 \pm 0.32$ ) having the lowest weight across the group. The control group was moderate with mean and standard error of mean ( $1.8 \pm 0.13$ ,  $1.9 \pm 0.13$ ) (Table 2, Figure 2).

**Table 3: The Distribution of Mean and standard error of mean (sem) for antioxidant enzymes**

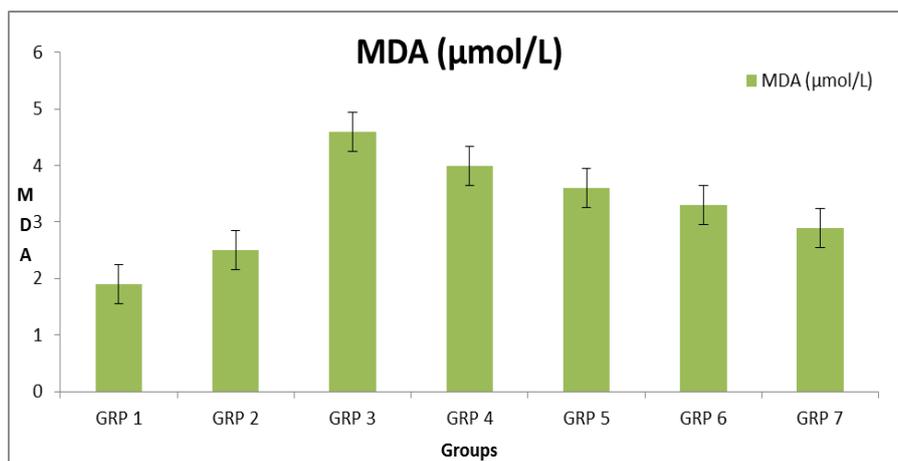
GROUPS	SOD(IU/ml)	GPx IU/L)	MDA ( $\mu\text{mol/L}$ )
GRP 1	$369.3 \pm 2.3$	$5907 \pm 29.1$	$1.9 \pm 0.18$
GRP 2	$352.7 \pm 1.5$	$5152 \pm 59.2$	$2.5 \pm 0.03$
GRP 3	$264.3 \pm 33.3$	$4028 \pm 11.7$	$4.6 \pm 0.13$
GRP 4	$259.0 \pm 3.8$	$4185 \pm 17.7$	$4.0 \pm 0.09$
GRP 5	$276.3 \pm 3.0$	$4482 \pm 23.5$	$3.6 \pm 0.09$
GRP 6	$300.0 \pm 2.9$	$4660 \pm 29.4$	$3.3 \pm 0.03$
GRP 7	$323.3 \pm 6.0$	$4868 \pm 42.9$	$2.9 \pm 0.06$



**Figure 3A: Variation in the Superoxide dismutase enzyme.**



**Figure 3B: Variation in the Glutathione Reductase enzyme.**



**Figure 3C: Variation in the Malondialdehyde enzyme.**

Statistically, the biochemical enzymes: Superoxide dismutase and Glutathione Reductase, Malondialdehyde were markedly significant ( $p < 0.05$ ). The mean and standard error of mean for Superoxide dismutase (SOD) are:  $369.3 \pm 2.3$ ,  $352.7 \pm 1.5$ ,  $264.3 \pm 33.3$ ,  $259.0 \pm 3.8$ ,  $276.3 \pm 3.0$ ,  $300.0 \pm 2.9$ ,  $323.3 \pm 6.0$ , (table 3, and figure 3A). Group 1 (Control) was the highest ( $369.3 \pm 2.3$ ) followed by group 2 (given *lawsonia inermis* alone) ( $352.7 \pm 1.5$ ) while the group with the lowest value was group 4 (Given lowest dose *lawsonia inermis* extract with aluminum chloride) ( $259.0 \pm 3.8$ ), table 3, figure 3A followed by group 3 (Given aluminum Chloride alone) ( $264.3 \pm 33.3$ ). According to table 3, figure 3B above, the mean and standard error of mean for Glutathione Reductase are:  $5907 \pm 29.1$ ,  $5152 \pm 59.2$ ,  $4028 \pm 11.7$ ,  $4185 \pm 17.7$ ,  $4482 \pm 23.5$ ,  $4660 \pm 29.4$ ,  $4868 \pm 42.9$ ; Group 1 (control) has the highest value ( $5907 \pm 29.1$ ), while group 3 (Given aluminum chloride alone) has the lowest value ( $4028 \pm 11.7$ ).

Malondialdehyde were markedly significant ( $p < 0.05$ ), with the mean and standard error of mean ( $1.9 \pm 0.18$ ,  $2.5 \pm 0.03$ ,  $4.6 \pm 0.13$ ,  $4.0 \pm 0.09$ ,  $3.6 \pm 0.09$ ,  $3.3 \pm 0.03$ ,  $2.9 \pm 0.06$ ) (IU/L), see table 3, figure 3C. 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  $1.9 \pm 0.18$  (IU/L).

## DISCUSSION

The findings of the present study showed that *Lawsonia Inermis* aqueous leaves extract could serve as a major defense against aluminum induced oxidative stress on reproductive function. There were no statistically significant changes in the animal weights difference of the experimental animals in all the groups in week zero and the third weeks ( $p > 0.05$ ) but there were statistically significant changes in the animal weights of the experimental animals in all the groups in first and second weeks ( $p < 0.05$ ). The animals that were given low dose and high dose of *lawsonia inermis* with aluminum chloride have their weights increased according to dosage we can deduce from those results that increase in the dosage have positive influence on the weights. The

group that was given aluminum chloride alone drastically reduced in weights, the reason is not far-fetched aluminum caused oxidative stress and affects the weights of the animals.

The differences in the Testicular weight across the groups were not too much distinct. Nevertheless, group 6 right and left testis with high dose of *lawsonia inermis* with aluminum chloride and group 7 right and left testis with ascorbic acid and aluminum chloride have the highest weights (Table 2, Figure 2). This may be adduced to the ability of the extract to curtail aluminium toxicity, also is a major defense against oxidative stress or contribute to the nutrition of the animal's testicular injury in rats. This finding is in agreement with Wang<sup>[39]</sup>, a number of non enzyme factors also function as antioxidants in the testis.

The biochemical findings shows that Superoxide dismutase and Glutathione Reductase were markedly significant ( $p < 0.05$ ). In figure 3A, Superoxide dismutase (SOD) were highest in control group rats followed by the rats given *lawsonia inermis* alone while the rats given low dose *lawsonia inermis* extract with aluminum chloride have the lowest value (table 3, figure 3A). According to table 3, figure 3B above, Glutathione Reductase of the control has the highest value, while the rats given aluminum chloride alone has the lowest value. Statistically, the biochemical enzymes: malondialdehyde were markedly significant ( $p < 0.05$ ). The rats that was given aluminum chloride alone has the highest value while group 1 (control) has the lowest value according to Table 3.

In contrast with report of Dasgupta<sup>[10]</sup> Superoxide dismutase levels were higher, while Malondialdehyde level was lower with *Lawsonia inermis* treated group, in a bid to showed the inhibitor effect of *L inermis* on gastric and skin papillomatous tumors. Superoxide dismutase plays an important role in protecting the toxic effects of superoxide radical and catalyzes the conversion of superoxide radicals to hydrogen peroxide.

Glutathione Reductase a seleno-enzyme, plays a major role in the reduction of hydrogen peroxide and hydroperoxide to non-toxic products.<sup>[42]</sup> Oxidative stress and a failure of antioxidant defense system cause several sperm abnormalities and result in infertility. In agreement with Sainath<sup>[43]</sup> an imbalance between the reactive oxygen species generation and scavenging system might be one of the reasons for aluminum chloride in rats given aluminum chloride alone induces male reproductive toxicity. *Lawsonia inermis* can indirectly scavenge reactive oxygen species such as hydrogen peroxide through increased activity of glutathione peroxidase; therefore it may prevent the testicular injury produced by aluminum chloride.

Exposure to Aluminum chloride induced oxidative stress and leads to increase value of malondialdehyde in the rats given aluminum chloride while rats with high dose of aqueous *Lawsonia inermis* leaf extract and aluminum chloride also recorded low value of malondialdehyde. This finding is actually in agreement with some of these authors.

According to Darley-Usman<sup>[9]</sup> 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.<sup>[23]</sup> According to Lucesoli and Fraga<sup>[23]</sup>, Wellejus and Poulsen<sup>[40]</sup>, exposure to high concentrations of certain metals would cause oxidative stress and high metal doses increase oxidative damage and deplete antioxidants in the testes of rats. 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.<sup>[31]</sup> One of the major mechanisms behind metal toxicity has been attributed to oxidative stress. Papa and Skulachev<sup>[31]</sup> reported that, 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. Also reported that reactive oxygen species are small, oxygen-based molecules that are highly reactive because of unpaired electrons. The most prominent reactive oxygen species are the superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), and the hydroxyl ion ( $OH^\cdot$ ). Testicular oxidative stress plays a role in a number of conditions known to be detrimental to male fertility. According to Karlsson<sup>[20]</sup>; Dandekar<sup>[8]</sup> lipid peroxidation of sperm membrane is considered to be the key mechanism of ROS-induced sperm damage leading to infertility.

Lastly, concomitant administration of aluminum chloride and ascorbic acid markedly reduced the testicular lipid peroxidation Table 3. According to Kehrer, and Smith<sup>[21]</sup>

ascorbic acid (antioxidants) are constantly rendering free radicals harmless.

## CONCLUSION

*Lawsonia inermis* aqueous leaf extract protects the cells and organ systems of the body against reactive oxygen species harmless, a good antioxidant agent against oxidative stress induced by heavy metal toxicity. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals.

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