# Safety Evaluation of *Lawsonia inermis* on Physiological, Andrological and Haematological Parameters of Male Wistar Rats

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## ABSTRACT

The leaves of *Lawsonia inermis* Linn are used in the treatment of many diseases such as diabetes, poliomyelitis, measles and gynecological disorders such as menorrhagia, vaginal discharge and leucorrhoea. This study was designed to investigate the safety evaluation of *Lawsonia inermis* Linn leaves (acutely and sub-chronically) on physiological, biochemical and histopathological changes seen in Wistar rat. Acutely, female rats were divided into four groups (n=3) and treated as thus A (untreated control); B (1000 mg/kg); C (2000 mg/kg) and D (5000 mg/kg). Sub-chronically, 25 male Wistar rats were grouped into five (n=5). Groups: A (control), B (100 mg/kg); C (200 mg/kg); D (400 mg/kg) and E (800 mg/kg). *Lawsonia inermis* Linn leaves have a wide safety margin (>5000mg/kg) and no mortality or visible toxic reaction was observed in acute phase. *Lawsonia inermis* extract did not inhibit physiological weight gain, except the highest dose that caused some weight loss. Haematological result showed that PVC, RBC, haemoglobin and platelets had no significant (P>0.05) effect unlike white blood cell and differentials (neutrophils, lymphocytes and monocytes) which decrease significantly (P <0.05) across all the treated groups compared to untreated control. Serum chemistry showed a significant (P <0.05) decrease AST. ALT, ALP, creatinine, urea, Total protein and Total bilirubin had no significant (P <0.05) effects. Serum electrolytes; calcium ion, potassium ion, sodium ion and chloride ion had no significant (P <0.05) changes. *Lawsonia inermis* is safe at acute administered dosages while nephrotoxicity and spermiotoxicity may occur following subchronic administration.

**Keywords:** *Lawsonia inermis*, Weight, Haematology, Serum chemistry, Electrolytes, Andrological parameters

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## INTRODUCTION

Plants with medicinal activities are being explored worldwide especially in developing countries where drugs from medicinal plants are playing an important role in health care delivery system (Barliana *et al.,* 2014). These plants contain numerous compounds which serve as potential drug sources for human and animal disease management (Barliana *et al.,* 2014). In folk medical practices; the use of plants with medicinal potential plays a significant role in covering the basic health needs in the developing countries. Recently, there has been an increase in the interest of scientific community to explore the pharmacological actions of medicinal plant or to ascertain the claims made about them in the official books of Ayurveda (Kasture *et al.,* 2001).

Medicinal plants mostly used for treatment of particular diseases, on large scale are reported to be having serious side effects. Many drugs have originated from biologically active plant chemicals and their medicinal uses are attributed to various active chemicals found in them. Various Studies have reported the toxic activities exhibited by extracts of most plants which cannot be mimicked when using pure compound isolated from purified constituents of the plant (Philomena *et al.,* 2009). Safety and toxicity of medicinal plant is usually related to viewpoint of perception because edible foods that are considered relatively safe may possess constituents that could trigger serious allergic reaction. Reports have shown that food containing toxic constituents like alpha gliadin produced by gluten in wheat, cyanogenic glycosides in most fruits, thiocyanates from vegetables, alkaloids and lectins from soy beans may cause significant toxicity when consumed (Ernst, 2007).

*Lawsonia inermis* (henna plant) is a very useful medicinal plants in many parts of the world and the leaves powder have been used for staining hair, nails and beard (Chengaiah *et al.,* 2010). The leaves are used in the treatment of many diseases such as diabetes, poliomyelitis, measles among the Yoruba tribe of South Western Nigeria (Oladunmoye *et al.,* 2011). The seeds on the other have been reported to possess deodorant action and are used in most cases of gynecological disorders such as menorrhagia, vaginal discharge and leucorrhoea (Nawagish *et al.,* 2007).

Henna from *Lawsonia inermis* is widely used in the cosmetic industry as dyeing agent also in many parts of the world (Nawagish *et al.,* 2007). Reports show that methanolic root extracts of *Lawsonia inermis* is used in Nigeria for cosmetic purposes and antimalarial (Idowu *et al.,* 2011) as well as for abortifacient purposes (Aguwa, 1997). The powdered of the roasted seed when mixed with ginger oil to form a paste is used in the treatment of ring worm. Decoction of the leaves is also used for aseptic cleaning of wounds and healing (Kumari *et al.,* 2013). *Lawsonia inermis* is also been used by some individuals as 'blood tonic', thus implying its multifaceted usage (Idowu *et al.,* 2011). Judging by all these potential benefits, this plant is not widely utilized. Hence this study carried out to establish the acute and sub-chronic toxic activities on weight, relative organ weight, haematology, biochemistry, electrolytes, andrological and histopathological changes.

## METHODS

### Plant Harvesting, Identification and Preparation

Leaves of *Lawsonia inermis* Linn was harvested from a farm land in Oke-oyi in Ilorin East area council of Kwara state, North Central, Nigeria. Taxonomically, it was both identified and authenticated at University of

Ibadan Herberium and a specimen was deposited and assigned a voucher number UIH-22460. The leaves of *Lawsonia inermis* Linn were dried at room temperature (25$^{o}$C) under shade in a room for four weeks. The leaves were macerated to powdery form using a blender with brand name Panasonic(R) Japan. The powdery leaves of *Lawsonia inermis* Linn was used for crude extracts.

**Extraction and Separation of *Lawsonia inermis Linn* Leaves**

Two kilograms of powdery leaves of the *Lawsonia inermis* was soaked in 5 liter of methanol for 72 hours. Mixture was gently decanted and filtered using filtered paper. The filtrate was immediately evaporated at temp 40°C using a rotary evaporator with brand name Buchhi(R). The concentrate (wet residue) was dried and stored 4$^{o}$C in the refrigerator branded LG.

**Experimental Animal and Ethical Consideration**

Adult Wistar rats (male and female) obtained from Experimental Animal House, Faculty of Veterinary Medicine, University of Ibadan, Ibadan and were used for this study. This work was ethically approved by ACUREC who is the regulatory body in charge of animal use in University of Ibadan. ACUREC issue a full approval with assigned number: UI-ACUREC/18/0063. All stress factors such as handling, feeding, housing, environmental conditions were adequately provided and the animals were humanly handled.

**Phytochemical Screening**

Dry solid samples of crude methanolic extract were assayed for phytochemical content following the methods described by Trease and Evans (1989).

**Acute Toxicity**

Acute toxic effect of crude methanol extract of *L. inermis* Linn. leave was carried out following the method of Organization for Economic Co-operation and Development (OECD) guideline 425. In this experiment, twelve female rats were divided into four (4) groups (n=3). The first group was administered with distilled water and served as control while the remaining three groups were administered with crude methanolic extract of *Lawsonia inermis* Linn. leave at 1000, 2000 and 5000 mg per kilogram body weight respectively.

Distilled water and *L. inermis* Linn leave was given to rats using oral gavage with canular. All the treated rats were monitored for behavioural changes, toxicity signs and death after 2-5 hours, then over 24 hours period.

**Sub-chronic Toxicity**

Twenty-five male rats were used for this phase; five rats per group (n=5). Four of the groups were administered crude methanol extract of *Lawsonia inermis* Linn leaves at 100, 200, 400 and 800mg/kg for fourteen days. One of the groups is the control and they were treated with distil water. The administration was done orally using oral gavage daily for 14 days. Rats were thereafter sacrificed and organ and blood samples were collected.

**Weighing of Rats and Their Organs**

All experimental rats were weighed before the start of the experiment and thereafter on weekly basis until last day of the experiment. The organs were weighed with electronic balance (Golden Metler(R)) and relative organ weight calculated

Relative organ weight (%) = [(Weight of the organ x 100) x Final Body weight$^{-1}$]

**Blood Sample Collection**

On fifteenth day, rats were anaesthesized using ether and haematological samples was collected from the median canthus of experimental animal for haematological and biochemical assays. Approximately 2ml each of whole blood sample was collected into both plain and EDTA bottles for heamatological screening. The serum was separated from the clot and centrifuged (3000 revolution per minutes (rpm) for 20 minutes) into Eppendorf tubes for biochemical assay.

***Haematological parameters***

The whole blood in the EDTA bottles were used in evaluation of haematological parameters. The parameters were evaluated using Cole's method (Cole, 1986); PCV, Hb Conc, and RBC. Others includes WBC, lymphocytes, monocytes and neutrophils were determined using Automatic analyzer (Auto Hematology Analyzer, China).

**Serum Biochemical and electrolytes Parameters**

Total protein (TP) and two of its constituent fractions; albumin (Alb) and Globlin (Glb), creatinine (CRT), blood urea nitrogen (BUN) was determined according to the method of Duncan *et al.* (1994). Serum enzymes including alanine transferase (ALT), alanine phosphatase (ALP) and aspartate transferase (AST). Serum electrolytes assayed includes calcium ion, potassium ion, sodium ion and chloride ions. These were determined by standard method using kits. Randox Chemicals Netherlands.

**Andrological Analysis**

Sperm was extracted from all the rats and were be analyzed for morphology (abnormal sperm cell) and sperm characterization (volume count, motility and live/dead ratio) using standard method.

**Histopathological Procedures**

The liver, kidneys and testes were carefully removed from the experimental rats. These organs were fixed with formalin (10%) so as to preserve the structural and molecular component. All fixated organs were dry out by bathing them in graded mixture of both ethanol and water. Ethanol was replaced with the embedding medium. Tissues were later infiltrated with xylene for clearing. Xylene impregnated tissue was placed in paraffin (embedding) inside an oven (Mermmet, Switzerland) and this was maintained at a temperature of 58 to 60$^{o}$C.

The generated heat will allow the solvent to evaporate creating space within the tissues so as to allow paraffin to fill the space. The paraffin will harden the tissue upon removal from the oven. 5 µm of the tissue was sectioned, floated in water and then transferred on to a glass slide. The sectioned tissues were stained with H&E. Stained and washed slides of various organs were viewed using light microscope at X100 magnification.

**Data Analysis**

All data generated were expressed as mean ±SD. The differences between the groups were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's post-hoc multiple comparison test using GraphPad Prism 5.03 statistical package, San Diego, California, U.S.A (www.Graphpad.Com). P value was considered significant at P≤ 0.05.

**RESULT AND DISCUSSION**

**Acute Toxicity of *Lawsonia inermis* Linn Leaves**

All the three experimental rats in various groups treated with crude

extract of *Lawsonia inermis* Linn was observed to be non-toxic at doses; 1000, 2000 and 5000mg/kg. No signs of systemic toxic effects and no mortality in all the treated groups. Thus, following Lorke's method (1983), the $LD_{50}$ of *Lawsonia inermis* Linn administered was observed to be greater than 5000mg/kg, and the extract was considered to be relatively safe.

**Table 1.** Phytochemical screening (qualitative) of methanol extract of *Lawsonia inermis* Linn leave

| **Test crude methanol extract** |
|---|
| Saponins ++ve |
| Tannins ++ve |
| Flavonoids ++ve |
| Cardiac glycosides ++ve |
| Terpenoids +ve |
| Steroids +ve |
| Anthraquinones +ve |
| Alkaloids +ve |

Interpretations-ve: Absent, +ve: Present, ++ve: Abundantly present

**Sub-chronic effects of *Lawsonia inermis* Linn leaves**

**Weight changes**

The extract did not inhibit physiological weight gain in the course of the study, except the highest (800 mg/kg) that caused some weight loss.

**Percentage Relative Organ Weight**

The mean weight of the heart, liver, kidney, testes and pancreas are vividly stated in the table below. All organs presented non-significant increased weight across the treatment groups except the kidney that increased significantly ($p<0.05$) in group dosed 800mg/kg of crude methanol extract of *Lawsonia inermis* Linn. The liver, testes and pancreas presented non-significant increase or decrease across the treatment groups compared to untreated control (Table 4).

**Spermatozoa Characterization and Morphology**

The normal sperm parameters of rats treated with various doses of *Lawsonia inermis* Linn showed that sperm motility decreased significantly ($p<0.05$) in groups dosed 200mg/kg of *Lawsonia inermis* Linn. Similar observation was seen in the sperm count of 400mg/kg which also decreased significantly ($p<0.01$). Sperm count followed the same trend showing significant decrease ($p<0.01$) in groups administered 200, 400 and 800mg/kg of *Lawsonia inermis* Linn compared to untreated group. Sperm volume and live/dead ratio showed no significant alteration in all the administered dosage compared them with the untreated control (Table 5).

**Sperm Morphology**

**Rudimentary tail**

Spermatozoa with rudimentary tail was slightly decrease in groups 200 and 800mg/kg while slight increase was seen in group treated at 200mg/kg. Group treated with 400mg/kg remain normal as seen in the untreated rats. The mean percentage was non-significant across all the tested group compared to untreated control rats (Table 6).

**Abnormal sperm**

The result of total abnormal sperm cell following administration of *Lawsonia inermis* Linn extract showed significant increase in both groups administered $200mgkg^{-1}$ ($p<0.01$) and $800mgkg^{-1}$ ($p<0.05$) compared to untreated rats. Both 100mg/kg and 400mg/kg showed non-significant increased ($p>0.05$) compared to untreated groups (Table 6)

**Tailless Head**

Control group had a greater number of tailless head abnormality compared to all other treatment groups Treatment 100mg/kg have slight increased (1.19±0.32 sperm cells/µL) percentage abnormality compared to the control (1.17±0.22%). The difference in the mean percentage abnormality were non-significant ($p>0.05$) compared to the extract treatment (Table 6).

**Headless Tail**

Groups treatment; 200mg/kg and 800mg/kg were noted with slight headless tail abnormality than those of the untreated group while other two groups (100 and 400mg/kg) have lesser abnormality compared to control group. Mean difference was non-significant across all group (Table 6).

**Bent tail**

All the experimental rat treatment (100, 400 and 800mg/kg) increased non-significantly in percentage abnormality of bent tail. But 200mg/kg presented significant increased ($p<0.05$) percentage abnormality compared to untreated control (Table 6).

**Curved tail**

The result for curved tail abnormality increases significantly ($p<0.05$) in group treated; 200mg/kg while other group present non-significant increased abnormality when compared to untreated groups (Table 6).

**Curved mid-piece**

Incidence of curved mid-piece spermatozoa abnormality presented a non-significantly increase in percentage abnormality in all treated groups compared to untreated control (Table 6)

**Bent mid-piece**

Population of sperm cell with bent mid-piece decrease significantly ($p<0.05$) in group administered 100 mg/kg compared to untreated control while other groups showed non-significant increase in percentage abnormality (Table 6).

**Looped tail**

The percentage abnormality of looped tail of spermatozoa was not significant as both the control and the treatment were less than 1%. The group administered 800mg/kg presented non-significant decreased percentage abnormality compared to untreated control. Other treatment groups increase non-significantly (Table 6).

**Haematology**

The haematology result showed that the PVC, RBC, haemoglobin (Hb) and platelets (PL) were statistically unchanged compared to untreated control rats. However, WBC and its differential count such as Neutrophils, Lymphocytes and Monocytes significantly ($p<0.05$) decreased across treated groups compared to untreated control. Platelet counts of treated rats decreased non-significantly ($p>0.05$) compared to untreated control rats (Table 7).

**Serum Biochemistry**

Rats administered *L. inermis* extract had non-significant decline in total protein, albumin, globulin, total bilirubin and creatinine levels compared to untreated control. ALT, ALP and urea levels of rats dosed the extract were non-significantly ($p<0.05$) increased compared to untreated control rats, particularly rats administered 800mg/kg with urea level showing significantly increased level (Table 8).

The serum electrolytes; calcium ion, potassium ion, sodium ion and chloride ion had no significant alteration in all the treatment groups except calcium ion at 800mg/kg that showed a

**Table 2.** Acute toxicity study in female Wistar rats 24-hours post administration of crude methanol extract of *Lawsonia inermis* Linn leaves

| Group (n=3) | Dose (mg/kg) | Dead rats (n) | Toxic signs observed |
|---|---|---|---|
| A | Distilled water | 0 | No toxic changes observed |
| B | 1000 | 0 | No toxic change noticed. |
| C | 2000 | 0 | Dullness, reduced feed |
| D | 5000 | 0 | Weakness, dullness, reduced feed |

**Table 3.** Weight changes over 14-days following sub-chronic administration of crude extract of *Lawsonia inermis* Linn leave to male Wistar rats

| Groups | Weight after administration (g) | Weight before administration (g) | Difference in weight (%) |
|---|---|---|---|
| (Control) | 173.6±1.22 | 168.4±1.61 | 1.90 |
| (100 mg/kg BW) | 142.6±3.85 | 130.6±13.50 | 3.95 |
| (200 mg/kg BW) | 111.6±8.26 | 106.5±5.91 | 2.41 |
| (400 mg/kg BW) | 138.8±4.50 | 133.8±2.76 | 2.09 |
| 800mg/kg BW) | 147.6±1.50 | 165.9±2.34 | -2.07 |

**Table 4.** Percentage relative organ weight (g) of Wistar rats administered methanol extract of *Lawsonia inermis* Linn leaves

| Grp/organ | Heart | Liver | Kidney | Testes | Pancreas |
|---|---|---|---|---|---|
| **Control** | 0.29±0.04 | 2.39±0.11 | 0.54±0.03 | 0.91±0.19 | 0.21±0.05 |
| **100mg/kg** | 0.31±0.02 | 2.35±0.17 | 0.56±0.06 | 0.99±0.06 | 0.19±0.04 |
| **200mg/kg** | 0.32±0.04 | 2.58±0.22 | 0.54±0.03 | 0.96±0.11 | 0.18±0.04 |
| **400mg/kg** | 0.30±0.05 | 2.29±0.21 | 0.61±0.02 | 1.00±0.06 | 0.19±0.06 |
| **800mg/kg** | 0.32±0.02 | 2.13±0.17 | **0.66±0.04**[a] | 1.11±0.07 | 0.19±0.02 |

Data rep. as Mean ±SD: n=5
[a] Significant at p≤0.05 compared to control rats

**Table 5.** Spermatozoa characterization of Wistar rats administered methanol extract of *Lawsonia inermis* Linn leaves

| Grp/index | Sperm motility (%) | Sperm count (x10⁶/µL) | Sperm volume (cm³) | Live/dead ratio (%) |
|---|---|---|---|---|
| **Control** | 92.50±2.89 | 137.00±8.60 | 5.18±0.05 | 98±0.01 |
| **100mg/kg** | 80.00± 0.01 | 123.30±6.08 | 5.18±0.05 | 96.50±1.73 |
| **200mg/kg** | 72.50±5.00[a] | 95.00±8.04 [b] | 5.18±0.05 | 96.50±1.73 |
| **400mg/kg** | 75.00±5.77[b] | 100.00±8.45 [b] | 5.18±0.05 | 96.50±1.73 |
| **800mg/kg** | 66.67±5.77 | 94.67±5.77 [b] | 5.20±0.02 | 96.00±1.73 |

Data rep. as Mean ±SD: n=5
[a b] Significant [a]p≤0.05 [b]p≤0.01

**Table 6.** Sperm morphology of Wistar rats administered methanol extract of *Lawsonia inermis* Linn leaves

| Grps/index | Control | 100mgkg$^{-1}$ | 200mgkg$^{-1}$ | 400mgkg$^{-1}$ | 800mgkg$^{-1}$ |
|---|---|---|---|---|---|
| **Total abnormal** | 11.25±0.96 | 12.50±0.58 | **14.5±1.29$^{b}$** | 12.75±0.96 | **13.67±1.16$^{a}$** |
| **Rudimentary tail** | 0.55±0.23 | 0.50±0.20 | 0.56±0.24 | 0.55±0.23 | 0.49±0.01 |
| **Tailless head** | 1.17±0.22 | 1.19±0.32 | 1.17±0.32 | 1.11±0.33 | 1.06±0.27 |
| **Headless tail** | 1.17±0.31 | 1.12±0.32 | 1.24±0.19 | 1.05±0.23 | 1.39±0.17 |
| **Bent tail** | 1.98±0.21 | 2.43±0.24 | **2.72±0.24 $^{a}$** | 2.34±0.38 | 2.38±0.53 |
| **Curved tail** | 2.04±0.28 | 2.31±0.30 | **2.78±0.27 $^{a}$** | 2.41±0.30 | 2.46±0.29 |
| **Curve mid-piece** | 2.22±0.45 | 2.31±0.25 | 2.59±0.20 | 2.41±0.28 | 2.79±0.16 |
| **Bent mid-piece** | 2.16±0.28 | 1.99±0.20 | 2.91±0.35$^{b}$ | 2.89±0.24 | 2.54±0.09 |
| **Looped tail** | 0.55±0.23 | 0.56±0.24 | 0.62±0.25 | 0.55±0.23 | 0.49±0.25 |

Data rep. as Mean ±SD: n=5
$^{a\ b}$ Significant $^{a}$p≤0.05 $^{b}$p≤0.01

**Table 7.** Haematology of Wistar rats administered methanol extract of *Lawsonia inermis* Linn leaves

| Grps/index | Untreated Control | 100mgkg$^{-1}$ | 200mgkg$^{-1}$ | 400mgkg$^{-1}$ | 800mgkg$^{-1}$ |
|---|---|---|---|---|---|
| PCV (%) | 37.25±1.71 | 38.75±4.11 | 36.00±1.83 | 38.00±4.76 | 35.00±2.83 |
| RBC×10$^{6}$/ µL | 6.16±0.42 | 6.49±0.72 | 6.00±0.32 | 6.23±0.74 | 5.94±0.42 |
| HB (g/dl) | 11.20±0.83 | 11.75±1.17 | 10.93±0.51 | 11.53±1.56 | 10.80±0.71 |
| MCV (fl) | 59.75±1.50 | 59.25±0.50 | 59.0±0.82 | 61.0±1.41 | 60.0±0.00 |
| MCH (pg) | 18.13±0.21 | 18.08±0.26 | 18.18±0.09 | 18.40±0.39 | 18.45±0.07 |
| MCHC (g/dl) | 30.00±0.91 | 30.30±0.36 | 30.23±0.21 | 30.23±0.74 | 30.90±0.36 |
| WBC×10$^{3}$/µl | 7.80±1.68 | **2.63±0.21$^{c}$** | **4.03±0.68$^{a}$** | **4.05±2.10 $^{a}$** | **3.76±2.33 $^{a}$** |
| Lymph×10$^{3}$/µl | 3.67±1.03 | **1.12±0.11 $^{b}$** | **2.03±0.55 $^{a}$** | **2.15±0.97 $^{a}$** | **1.84±1.22 $^{a}$** |
| Mono×10$^{3}$/µl | 0.09±0.01 | 0.04±0.02 | 0.06±0.03 | 0.03±0.02 | 0.06±0.08 |
| Platelet×10$^{5}$/dl | 2.20±0.68 | 18.80±6.81 | 16.28±9.22 | 17.65±5.44 | 21.90±0.57 |

ata rep. as Mean ±SD: n=5
$^{a\ b\ c}$ Significant $^{a}$p≤0.05 $^{b}$p≤0.01 $^{c}$p≤0.001

**Table 8.** Serum biochemistry of Wistar rats administered methanol extract of *Lawsonia inermis* Linn leaves

| Grps/index | Untreated Control | 100mgkg$^{-1}$ | 200mgkg$^{-1}$ | 400mgkg$^{-1}$ | 800mgkg$^{-1}$ |
|---|---|---|---|---|---|
| **T. Protein (g/dl)** | 7.65±0.71 | 6.34±0.16 | 6.94±0.51 | 6.73±0.49 | 6.40±0.31 |
| **Albumin (g/dl)** | 5.50±0.44 | 4.28±0.45 | 4.50±0.40 $^{b}$ | 5.50±3.11 | 4.50±2.12 |
| **Globulin (g/dl)** | 2.15±0.32 | 2.06±0.11 | 2.44±0.14 | 1.23±0.32 | 1.90±0.88 |
| **T. Bil. (µmol/l)** | 1.85±0.31 | 1.53±0.22 | 1.51±0.15 | 1.70±0.20 | 1.69±0.21 |
| **ALT (mmol/l)** | 29.08±6.17 | 31.40±5.94 | 31.73±2.79 | 30.55±4.49 | 32.60±5.66 |
| **ALP (mmol/l)** | 35.75±6.33 | 36.30±4.42 | 38.65±4.86 | 34.50±11.93 | 39.75±4.03 |
| **Urea (mmol/l)** | 2.80±0.14 | 2.93±0.46 | 2.83±0.43 | 2.65±0.31 | **3.70±0.42$^{a}$** |
| **Creatinine (µmol/l)** | 58.40±6.53 | 58.40±6.53 | 57.35±8.76 | 54.70±8.41 | 49.15±3.32 |

Data rep as Mean ±SD: n=5
$^{a\ b}$ Significant $^{a}$p≤0.05 $^{b}$p≤0.01

**Table 9.** Serum electrolytes (mmol/l) of Wistar rats administered methanol extract of *Lawsonia inermis* Linn leaves

| Grp/index | $K^+$ | $Cl^-$ | $Na^+$ | $HCO_3^-$ | $Ca^{2+}$ |
|---|---|---|---|---|---|
| **Control** | 1.73±0.38 | 64.08±24.40 | 98.30±27.78 | 21.50±1.92 | 0.53±0.17 |
| **100mg/kg** | 2.00± 0.67 | 61.40±12.14 | 96.60±13.28 | 20.01±3.20 | 0.50±0.12 |
| **200mg/kg** | 2.45±0.54 | 63.23±7.53 | 88.70±14.11 | 19.03±3.83 | 0.60±0.29 |
| **400mg/kg** | 2.73±3.35 | 60.55±14.27 | 80.00±17.09 | 18.50±3.11 | 0.65±0.19 |
| **800mg/kg** | 2.40±0.57 | 62.60±5.66 | 84.75±11.10 | 22.20±1.41 | 0.61±0.07 |

Data rep as Mean ±SD: n=5
[a b] Significant [a]p≤0.05 [b]p≤0.01

**Histopathology of the liver of Wistar rats administered methanol extract of *Lawsonia inermis* Linn leaves**

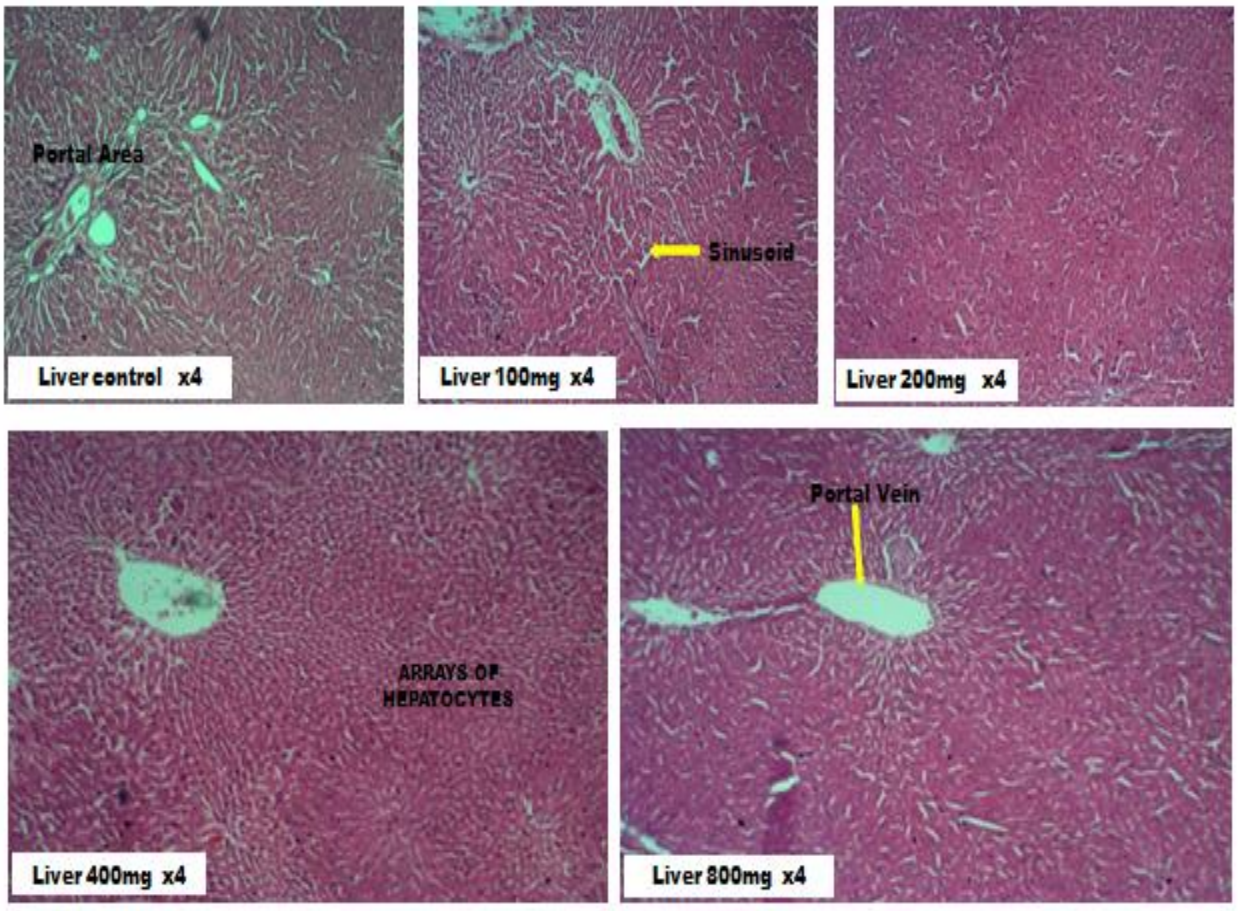



**Plate i-v:** Histomorphological presentation showing panoramic views of adult rat liver (H&E). Comparative observation across the micrographs shows a well outlined arrays hepatic cells and vessels, without any observable cytoarchitectural distortion in sample treated 100mg, 200mg, 400mg and 800mg (H&E) x40

**Histopathology of the kidney of Wistar rats administered methanol extract of *Lawsonia inermis* leaves**

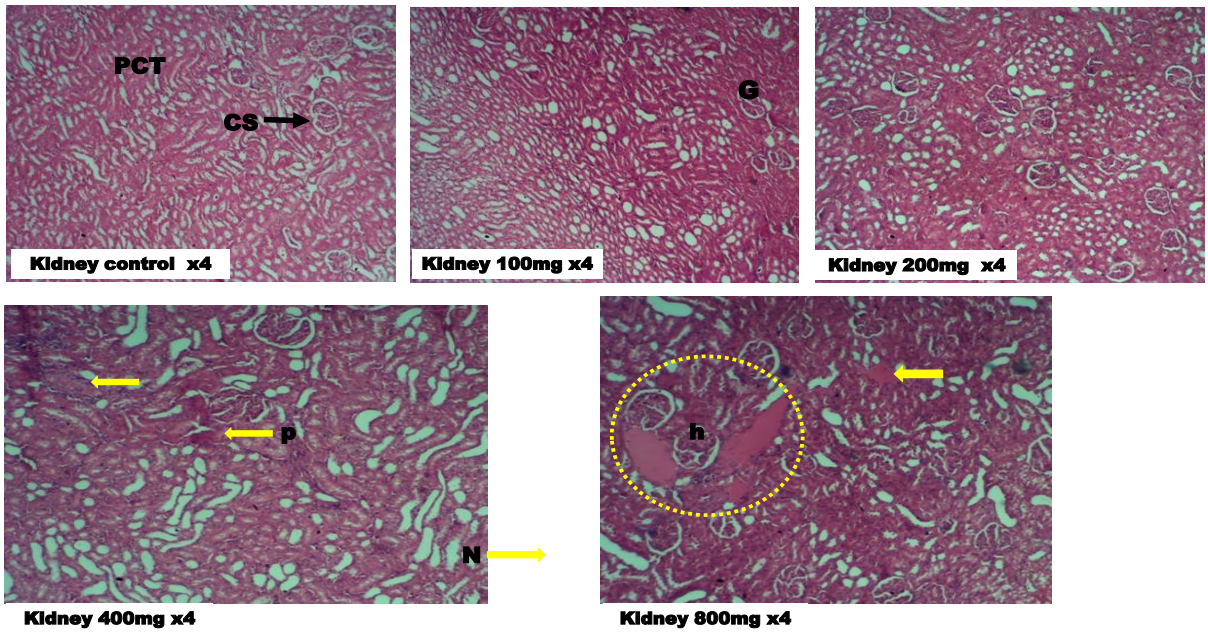
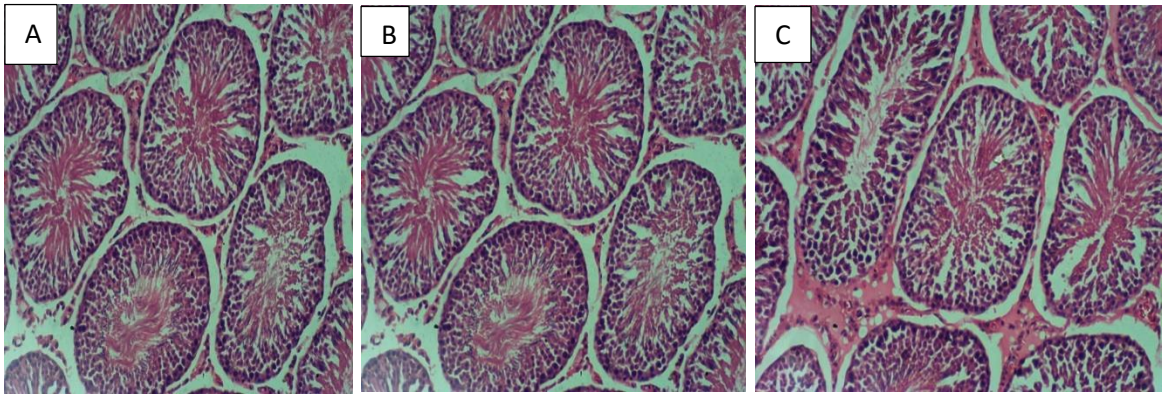
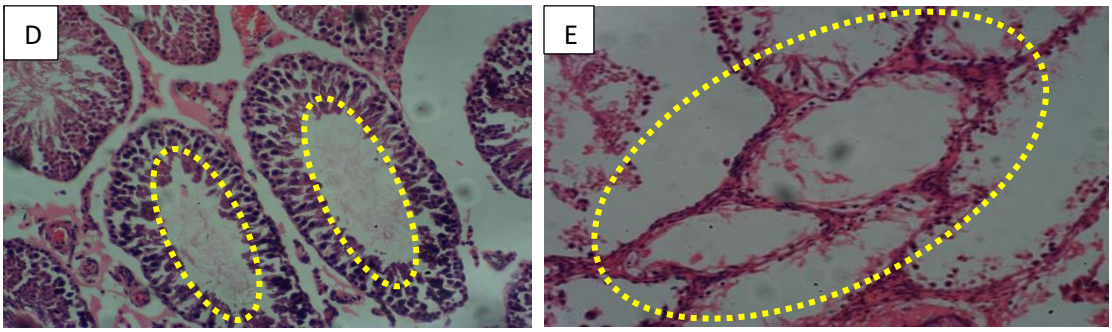



**Plate vi-x:** Comparative observation across the micrographs shows a well demonstrated renal outlines with intact Glomeruli, Renal tubules and adequate Capsular space. No significant cell distortion observed in Experimental Groups 'Control', '100mg' and '200mg'. Treatment Group 400mg shows mild dilation of the renal tubules, characterized with mild interstitial polymorph nuclear infiltrations (p). Focal areas of necrotic (n) tissues were also seen. However, the glomeruli were intact with no significant cellular distortion. Treatment group 800mg was characterized with marked interstitial deposition of eosinophilic materials (h) and multifocal tubular dilation. Mild Degenerative changes and necrotic areas were also observed. (H and E X40).

**Histopathology of the testes of Wistar rats administered methanol extract of *Lawsonia inermis* leaves**






(A) Untreated control: Photomicrograph of testes x400 showing No visible lesion H&E x400
(B) 100 mg/kg) 100mg/kg: testes showing No visible lesion H&E x400
(C) 200 mg/kg: Photomicrograph of testes showing No Visible lesion H&E x400
(D) 400mg/kg: Testes showing testicular atrophy and erosion testicular atrophy (doted circles)
(E) 800mg/kg: Photomicrograph of testes showing severe testicular degeneration (star) and germ cell depletion HE x400 (doted circle)

significant ($p<0.01$) increased values compared to untreated control (Table 9). Medicinal activities of most aromatic plant are due to existence of active constituents like alkaloids, tannins, phenols and flavonoids. Different medicinal plant has its distinct set of secondary metabolites which are the basis for novel drug discovery. Extensive reports have shown that these constituents (glycosides, triterpenes, flavonoids, monoterpenes and glycosides) in various solvents are accountable for most pharmacological properties (Hussain *et al.,* 2011).

Phytochemical analysis of crude methanol extract of *Lawsonia inermis* Linn. leaves used in this study showed presence of major constituents like Flavonoids, Anthraquinones, Alkaloid, Saponin, Tannins and Steroidal glycosides. These observed constituents agree with Khan and Nasreen that confirmed the phytochemical constituent of *L. inermis* Linn leaves (Khan and Nasreen, 2010). Phytochemical analysis of crude methanol extracts reveals that there exist a broad group of secondary constituents and this may be accountable for multifaceted activities of the plant (Khan and Nasreen, 2010). Saponin, tannins, flavonoids and cardiac glycosides were the four abundant phytochemical constituents observed in the crude extract. Saponin are often bitter to taste leading to reduce palatability but it is interestingly known to enhance nutrient absorption and smooth digestibility in animals (Kumari *et al.,* 2013). Tannins are plant polyphenol with extensive anti-oxidant activities and it has been reported for its ant-inflammatory potential. Flavonoids on the other hand possess significant health benefits due their antioxidant activities usually linked to functional hydroxyl groups that scavenge free radical and chelation of metallic ions (Khan and Nasreen, 2010). Cardiac glycosides are natural drug with primary effects on the heart in form of benefit (cardiotonic) and toxicity (heart poisons). Cardiac glycoside is beneficial when it increases the force of contraction of the cardiac muscle during arrythmias and cardiac failure (Menger-Schulz *et al.,* 2013).

The $LD_{50}$ of crude methanol extract of *L. inermis* Linn leaves in Wistar rats were not determined in this study, as the highest dose (5000 $mgkg^{-1}$) administered did not cause significant toxicity. This result showed that *L. inermis* Linn leaves had a wide safety margin and can be classed as a "Class IV" compound. General result of toxicity study showed that *Lawsonia inermis* Linn leaves is a safe plant and this was in agreement with Mudi *et al.* who reported aqueous root extract of *L. inermis* Linn did not cause mortality in Wistar rats. The study further indicated that *L. inermis* root extract leads to delayed toxic clinical manifestation such as paralysis, anorexia and weakness following intraperitoneal injection of the extract (Mudi *et al.,* 2011). The outcome of this present study did not show any abnormal clinical manifestation because leaves extract of *L. inermis* was used as against the root employed by Mudi *et al.* (2011) (Mudi *et al.,* 2011).

Medicinal plants have to undergo safety/toxicity evaluation in different organ/systems using various experimental techniques prior to pharmacological studies which assess their efficacy and these toxicity reports entail the effect(s) of the substance on the organs, blood or tissue (Ekor, 2013). Most reports on toxicological research lay emphases on hepatotoxicity associated with medicinal plant use (Ekor, 2013). Other organs that may be affected include kidney, heart, pancreas, testes and spleen. Reports have shown that *L. inermis* Linn possesses protective effect on the liver, kidneys and heart. Other pharmacological and modulatory activities include anti-inflammatory, antibacterial, antiviral, antifungal, antioxidant, analgesic and adaptogenic potential rendering it a safe plant which can be used as source of drug (Spellberg *et al.,* 2008).

Relative organ weight of liver, heart, testes and pancreas were not affected in all the extract-treated rats except in rats administered the highest dose (800mg/kg) in the subchronic toxicity study. Significant increase in the kidney weight may be indicative of a level of nephrotoxicity in these rats. This observation was corroborated by mild degenerative and necrotic changes seen in histopathology of the kidney tissues (Plate i-v). Organomegaly is a sign of disease, pathological abnormality or toxicity from drugs and other toxic substances. Standard and accepted table defining organomegaly is yet to be established (Molina *et al.,* 2015). Organ-somatic ratio is an important index of inflammation, atrophy and hypertrophy (Moss and Henderson, 2010).

The blood picture is usually evaluated in toxicity assessments and this current study showed that most blood indices; PVC, RBC, haemoglobin, MCV, MCH, MCHC and platelet counts presented non-significant changes, except WBC and its differential count (lymphocytes, monocytes and neutrophils) which decreased significantly across treatment groups. General haematology result of this toxicity study showed that crude extract of *L. inermis* Linn leaves exhibited significant improvement on most haematological parameters. This result agrees with Nostro *et al.* (2012) stating that most medicinal plants improve haematological indices. WBC on the other decreased significantly across the treated rats and this can lead to decreased immunity. This observation agrees with Nostro *et al.* (2012) that pointed out that crude extract of *L. inermis* Linn. caused significant alteration in white blood cells (Nostro *et al.*, 2012).

Serum levels of biochemical metabolites provide useful information on the toxicity or safety of therapeutic

substances including medicinal plants (Kiessoun *et al.,* 2012). Hepatocellular damage is specifically determined by elevation of serum enzymes that had leaked into the serum from the liver (Umar *et al.,* 2010). Serum chemistry of rats in this study presented non-significant reduction in AST levels, while ALT, ALP, creatinine, urea, Total protein and total bilirubin had no significant changes. This observation agrees with John *et al.* that reported varying alteration in hepatocellular enzymes following administration of *L. inermis* Linn leaves extract to Wistar rats (John *et al.,* 2020).

This present study showed that no significant change was observed in calcium ion, potassium ion, sodium ion and chloride ion levels, indicating that *L. inermis* Linn did not alter the delicate electrolyte balance and homeostasis in biological systems, further emphasizing its safety. This observation agrees with Agabna *et al.* (2014) who reported that ethanol extract of *L. inermis* Linn. seed does not alter serum electrolytes [Agabna *et al.,* 2014). Electrolytes are important indices used clinically to determine the function of kidney (Burton, 2004). Increased or decreased level of various electrolytes may be used as a marker pointing to renal insufficiencies. In diarrhearic situation, most of the electrolytes are lost in fluid. During fluid loss (dehydration), the concentration of ions balance may be altered and this alteration may shift to favourable conditions (Odutola, 2012).

Sperm dysfunction is the main cause of male sterility and one of the target tissues for toxicity in biological systems is the reproductive system, with emphasis on the architecture of the testes and sperm parameters (Cyrus *et al.,* 2015). Sperm motility is reported to be a determining factor for the success of fertilization, naturally and experimentally. Sperm motility levels are directly correlated with ability of a fertile male to achieve fertilization and conception in a fertile female (Zhou *et al.,* 2008). The concentration and virility of sperm cells to reach fertilization site are important determinant factor of fertility (Liu *et al.,* 2018). This present study showed that normal motility and count decreased significantly in all treated rats and this showed reproductive toxicity potential that may adversely affect the fertility of male animals. This observation may give credence to male contraceptive potential of *Lawsonia inermis* Linn which has been reported by Munshi *et al.* (2007). The sperm volume and live-dead ratio had no significant alteration in all treatment groups. Total abnormal sperm parameters increased at the highest dose used (800mg/kg) in relation to all other treatment groups. This observation agrees with work of Cyrus *et al.* which reported that extracts of most medicinal plant had adverse reproductive effects (Chaudhary et al., 2012).

## CONCLUSION

Conclusively, this study showed that *Lawsonia inermis* is safe at acute administered dosages because there is absence of mortality. Nephrotoxicity and spermiotoxicity may occur following subchronic administration. The safest among the studied doses is 100mg/kg therefore should be taken into consideration whenever the plant is being use for therapeutic purposes.

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