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EFFECT OF AQUEOUS LEAF EXTRACT OF *LAWSONIA INERMIS* ON LEAD ACETATE-INDUCED LIVER IMPAIRMENT IN WISTAR RATS

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ABSTRACT

Background: Lead is one of the most hazardous and persistent metal in the world. It has been implicated in several conditions. *Lawsonia inermis*, on the other hand, has been reported to possess hepatoprotective activity, amongst others. The goal of this study was to investigate the effect of aqueous leaf extract of *Lawsonia inermis* on lead acetate-induced liver impairment in Wistar rats. **Methods:** Thirty-five Wistar rats weighing between 167 and 204 grams were obtained from the University of Benin's Department of Anatomy in Benin City, and divided into seven groups of five animals each. Group A, B, C, D, E, F and G received distilled water (2 ml), 400 mg/kg aqueous extract of *L. inermis* only, 100 mg/kg of lead acetate only, 100 mg/kg of lead acetate and 70 mg/kg of Silymarin, 100 mg/kg of lead acetate and 200 mg/kg of *L. inermis*, 100 mg/kg of Lead acetate and 400 mg/kg of *L. inermis*, and 400 mg/kg of *L. inermis* and 100 mg/kg of Lead acetate, respectively. After treatment, blood and liver tissues were collected for analysis. **Results:** The administration of *L. inermis* extract protected the plasma membrane from lead acetate, and also increased the liver's regenerative and reparative capacity, as evidenced by significant reductions in serum ALT, AST, ALP, and total bilirubin levels, in addition to results from the histopathological analysis. **Conclusion:** The hepatoprotective and ameliorative activity of *Lawsonia inermis* aqueous leaf extract against lead acetate-induced liver damage in Wistar rats was clearly observed as a result of this investigation.

KEYWORDS: Liver, *L. inermis*, lead acetate, hepatotoxicity, Wistar rats.

INTRODUCTION

Many modern medications have their roots in ancient herbal treatment (Muhammad and Muhammad, 2005). Natural products still make up the major health care system and are a substantial source of synthetic and traditional herbal medicine (Nayak et al., 2007). Traditional medicinal approaches, particularly the use of medicinal plants, continues to be important in meeting the basic health needs of poor countries. The scientific community's interest in studying the pharmacological effects of herbs has skyrocketed in recent years (Une et al., 2001). *Lawsonia inermis* is one of the pharmaco-relevant plants.

Lawsonia inermis is a member of the Lythraceae family. It is a dicotyledonous herbaceous plant that blooms every two (2) years. The plant, which is native to North Africa and South-West Asia, is now widely cultivated as an ornamental and dye plant across the tropics. It is a hairless shrub or small tree with many branches (2 to 6 m in height). Its l eaves are tiny, opposite in arrangement along the branches, sub-sessile, 1.5 to 5 cm long, 0.5 to 2 cm in width, greenish brown to dull green, elliptic to broadly lanceolate with whole border, petiole short, acute or obtuse apex with a base that tapers. Young branches of *Lawsonia inermis* are green and quadrangular in shape, turning crimson as they mature. When young, the bark is greyish brown and unarmed, but mature trees' branches have spines. A big pyramid-shaped cyme is the inflorescence. Its flowers are small, about 1 cm across, many, fragrant, white or rose-colored with four (4) shattered petals, and they bloom in clusters. Its calyx has a 0.2 cm tube and 0.3 cm lobe spread. A little brown-colored spherical capsule serves as the fruit. At maturity, the fruit opens unevenly and separates into four (4) pieces, with many seeds. Seeds are around 3 mm in diameter, abundant, smooth, pyramidal, hard, thick seed coat with brownish coloring (Sastri, 1962; Vasudevan and Laddha, 2003; Chauhan and Pillai, 2007).

Different parts and extracts of *Lawsonia inermis* have been reported to possess several pharmacologic properties. For example, the ethanol leaf extract (Syamsudin and Winarno, 2008) and methanol leaf extract (Arayne et al., 2007), have demonstrated antidiabetic activity, methanol leaf extract (Mikhaeil et

al., 2004), and naphthoquinone fraction from leaves (Dikshit et al., 2000) – immunomodulatory activity, ethanol leaf extract (Dasgupta et al., 2003) and chloroform leaf extract (Endrini et al., 2007) – antioxidant activity, ethyl acetate leaf fraction (Ali et al., 2001) and aqueous leaf extract (Baba-Moussa et al., 1997) – antibacterial activity, bark extract (Singh and Pandey, 1989), and ethanol, methanol and aqueous leaf extract (Natarajan and Lalithakumar, 1987) – antifungal activity, ethanol fruit extract (Khan et al., 1991) – antiviral activity, methanol leaf extract (Wurochekke et al., 2004) – antitrypanosomal activity, ethyl acetate, ethanol and hexane fractions (Natarajan et al., 2000) – antidermatophytic activity, ethanol seed extract (Munshi et al., 1977) – antifertility, ethanol leaf extract (Moshin et al., 1989) – analgesic activity, isolates from stem bark and root (Gupta et al., 1993) – anti-inflammatory activity, ethanol leaf extract (Eze and Akonoafua, 2019) and alcoholic bark extract (Ahmed et al., 2000) – hepatoprotective activity.

Lead is one of the most useful metals, but it is also one of the most hazardous (Shotyk et al., 2005). Lead is a xenobiotic, long-lasting toxic metal (Reglero et al., 2009) that has been linked to neurological, hematological, gastrointestinal, reproductive, circulatory, immunological, and hepatic diseases, all of which have been connected to the dose and duration of exposure (Ademuyiwa et al., 2007; Park et al., 2006; Patrick, 2006; Vega-Dienstmaier et al., 2006). The goal of this study was to investigate the effect of aqueous leaf extract of *Lawsonia inermis* on lead acetate-induced liver impairment in Wistar rats.

MATERIALS AND METHOD

Plant material

The leaves of *L. inermis* were obtained from Uselu Market in Egor Logal Government area, Edo State, Nigeria. The plant was identified and authenticated by a plant taxonomist at the department of Plant Biology and Biotechnology, faculty of Life sciences, University of Benin, Benin city, Edo State, Nigeria.

Preparation of plant extract

Cold water maceration was used to obtain the plant's aqueous extract. The leaves of *L. inermis* were washed in tap water, shade-dried, and then pulverized. In a separating funnel, the powder was steeped in distilled water for twenty-four (24) hours with intermittent shaking. After then, the solution was filtered. After allowing the filtrate to settle, it was decanted. The decant was placed in an evaporating dish and then placed in a water bath at 60 °C to allow the surplus water to evaporate slowly, following which the dried residue was scraped out and kept in the refrigerator.

Experimental animals

Thirty-five Wistar rats weighing between 167 and 204 grams were obtained from the University of Benin's Department of Anatomy in Benin City, Edo State, and divided into seven (7) groups of five (5) animals each. The animals were acclimatized in the Department of Anatomy's animal house for two (2) weeks. They were kept in hygienic conditions in well-ventilated aluminum cages at room temperature and fed a conventional laboratory meal. Food and drink were freely accessible.

Induction of hepatotoxicity

The administration of 100 mg/kg body weight of lead acetate for 28 days caused hepatotoxicity. This was carried out in accordance with previously established procedures (Alabbassi et al., 2008; Osifo et al., 2015).

Experimental design

GROUPS	DOSAGE
GROUP A:	Received distilled water (2 ml) for twenty-eight (28) days
GROUP B:	Received 400 mg/kg aqueous extract of *L. inermis* only for twenty-eight (28) days
GROUP C:	Received 100 mg/kg of lead acetate only for twenty-eight (28) days
GROUP D:	Received 100 mg/kg of lead acetate and 70 mg/kg of Silymarin for twenty-eight (28) days simultaneously.
GROUP E:	Received 100 mg/kg of lead acetate and 200 mg/kg of *L. inermis* for twenty-eight (28) days, simultaneously.
GROUP F:	Received 100 mg/kg of Lead acetate and 400 mg/kg of *L. inermis* for twenty-eight (28) days, simultaneously.
GROUP G:	Received 400 mg/kg of *L. inermis* and 100 mg/kg of Lead acetate after 30 minutes for twenty-eight (28) days

Sacrifice, sample collection, processing and staining

On the twenty-ninth (29th) day, the animals were sacrificed humanely using chloroform anaesthesia. The livers were extracted and stored in specimen-collecting bottles after a longitudinal incision was made in the abdominal cavity. Blood was drawn through heart puncture and deposited into plain bottles before being processed for examination. Excised liver sections were washed in cold phosphate buffer saline and then processed for tissue preparation according to standard histological procedures (Drury et al., 1976).

Photomicrography

The H&E stained liver slides were viewed by a histopathologist, using a Leica DM750 research microscope with a connected digital camera (Leica CC50). The tissues were digitally imaged at magnifications of x400.

Statistical analysis

The data was presented as Mean ± SEM (standard error of mean). The differences in the means were determined using one-way analysis of variance (ANOVA). The results were considered statistically significant if the P value was less than 0.05. The LSD Post Hoc test was performed. The statistical program SPSS version 20 (IBM Corporation, NY) for Windows was used to analyze the data acquired in the study.

RESULTS

Plate 1 is a photomicrograph of the liver of Group A; it shows the normal histoarchitecture of the liver comprising hepatocytes, sinusoids, portal vein and bile duct. Plate 2 is a photomicrograph of the liver of Group B; it shows normal hepatocytes, mild periportal infiltrates of inflammatory cells and Kupffer cell activation. Plate 3 is a photomicrograph of the liver of Group C showing pathologic features such as ulceration of the blood vessels, necrosis, periportal infiltrates of inflammation and vascular congestion.

Group D is represented by Plate 4 and it shows normal histoarchitecture of the liver: normal hepatocytes and Kupffer cell activation. Group E is represented by Plate 5 and it shows normal histoarchitecture of the liver: normal hepatocytes and Kupffer cell activation, with active vascular congestion. Plate 6 is a photomicrograph of the liver of Group F; it shows normal hepatocytes and Kupffer cell activation while Plate 7 is a photomicrograph of the liver of Group G showing periportal infiltrates of inflammatory cells, vascular congestion and patchy hepatocyte necrosis.

Table 2: Levels of liver function enzymes.

	Control	*L. inermis* only (400 mg/kg)	Lead acetate only	Lead acetate + Silymarin	Lead acetate + *L. inermis* (200 mg/kg)	Lead acetate + *L. inermis* (400 mg/kg)	*L. inermis* (400 mg/kg) + Lead acetate	P-value
ALP (µ/L)	162.00±7.55	158.00±30.02	201.67±79.14*	171.67±25.21	154.00±26.06	168.00±8.35	174.67±8.35*	0.009
ALT (µ/L)	49.67±2.91	46.33±4.37	63.33±3.76*	45.00±7.02	47.00±4.00	49.33±4.67	51.33±1.45	0.036
AST (µ/L)	135.00±4.62	125.33±7.44	210.33±26.27*	118.33±18.67	142.33±0.88	135.67±4.26	171.33±9.87*	0.002
Total bilirubin (mg/dl)	0.20±0.00	0.19±0.02*	0.13±0.03	0.20±0.06	0.13±0.03	0.10±0.00	0.13±0.03	0.022

*Significantly different from the control group at P<0.05
The results are mean of five rats in each group ± SEM

Photomicrographs

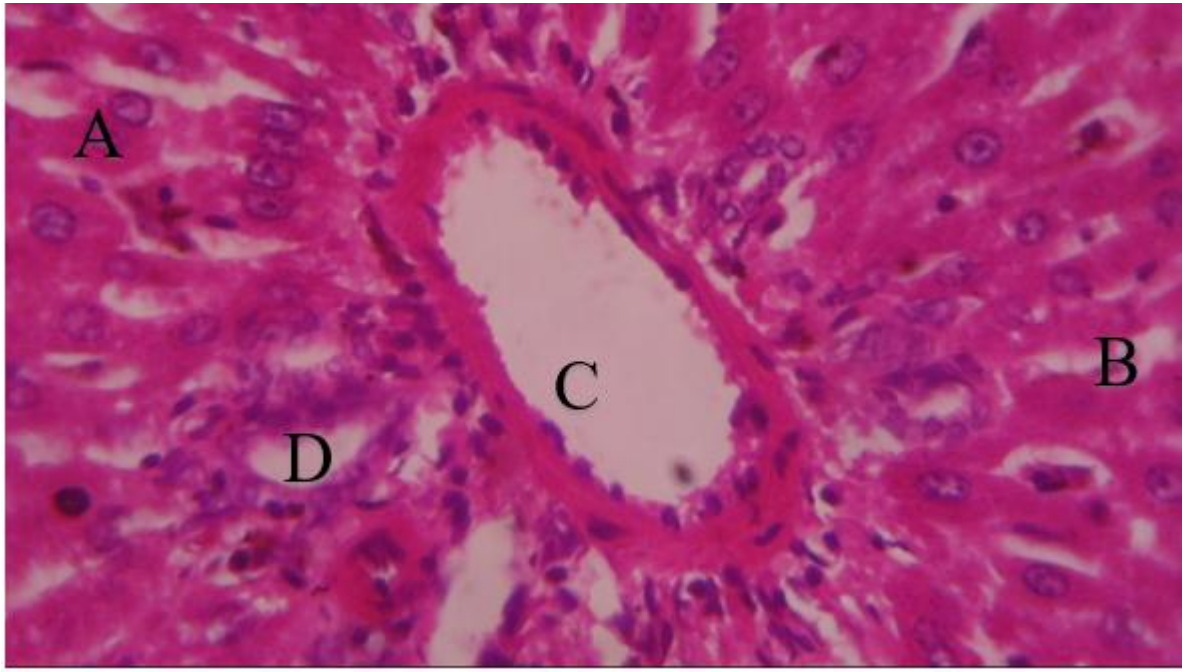

Plate 1: Control liver, composed of A: hepatocytes, B: sinusoids, C: portal vein and D: bile duct (H&E x 400).

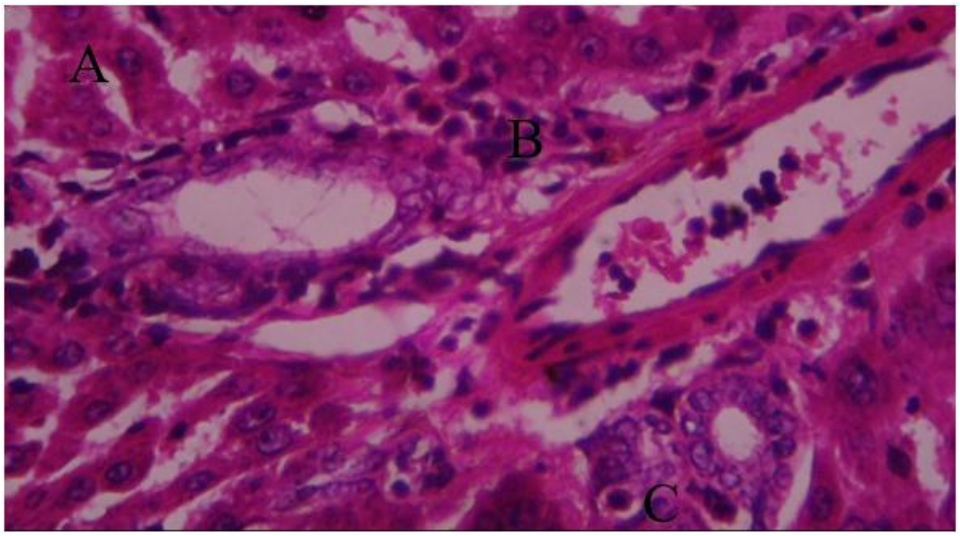

Plate 2: Rat given *L. inermis* only, showing A: normal hepatocytes, B: mild periportal infiltrates of inflammatory cells and C: Kupffer cell activation (H&E x 400).

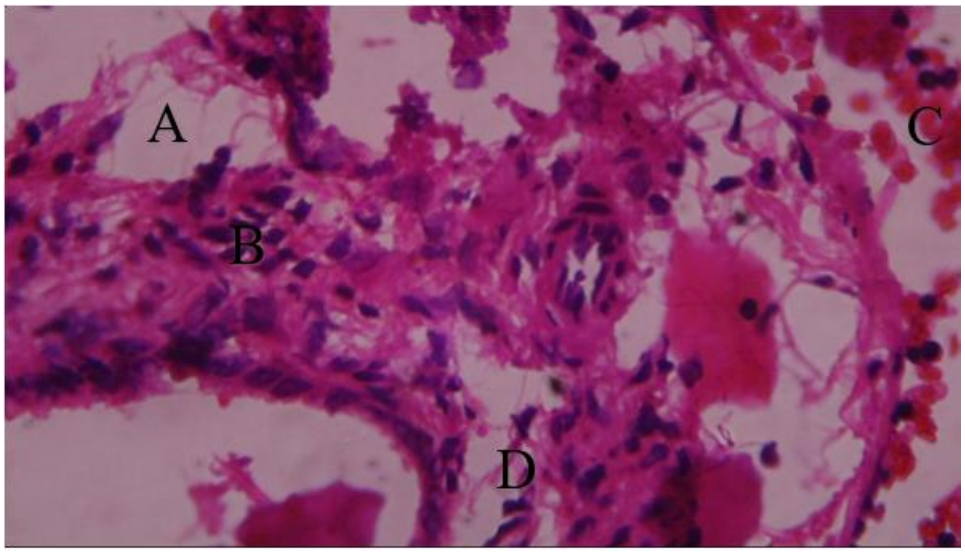

Plate 3: Rat given lead acetate only, showing A: vascular ulceration, B: periportal infiltrates of inflammation, C: vascular congestion and D: necrosis (H&E x 400).

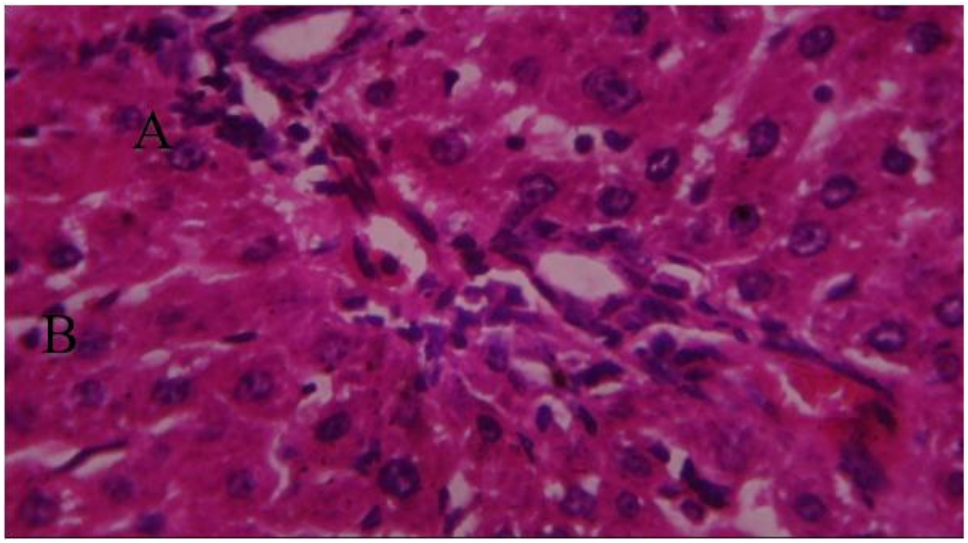

Plate 4: Rat given lead acetate + Silymarin, showing A: normal hepatocytes and B: Kupffer cell activation (H&E x 400).

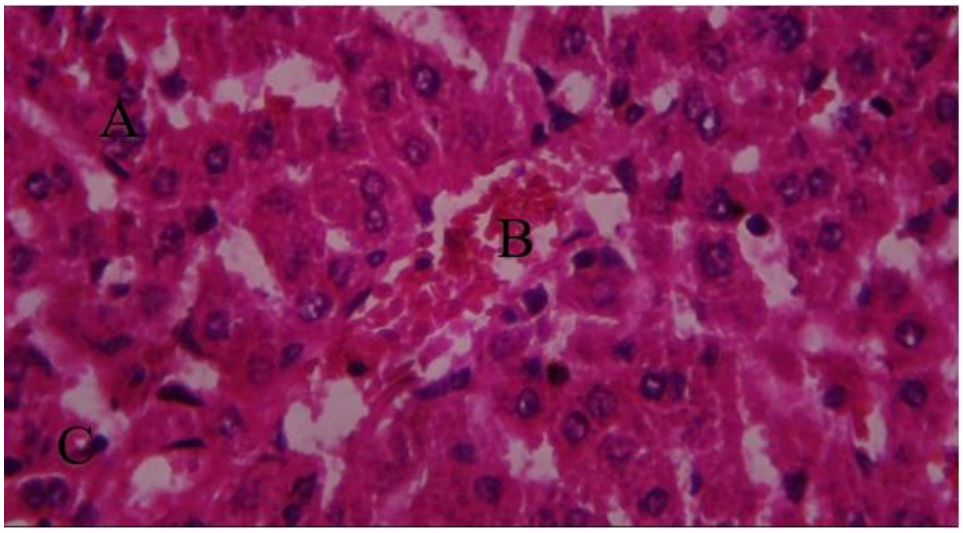

Plate 5: Rat given lead acetate + 200 mg/kg of *L. inermis* showing A: normal hepatocytes, B: active vascular congestion and C: Kupffer cell activation (H&E x 400).

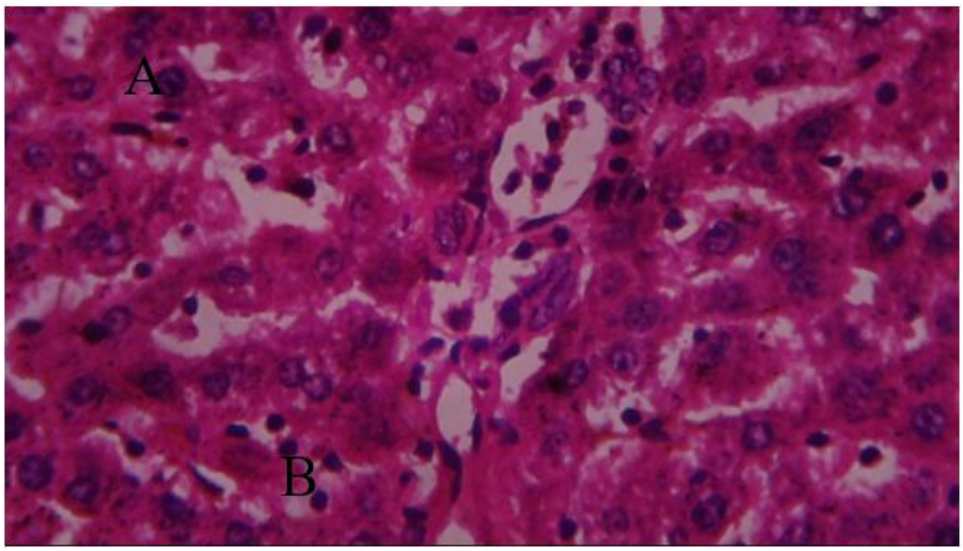

Plate 6: Rat given lead acetate + 400 mg/kg of *L. inermis* showing A: normal hepatocyte and B, Kupffer cell activation (H&E x 400).

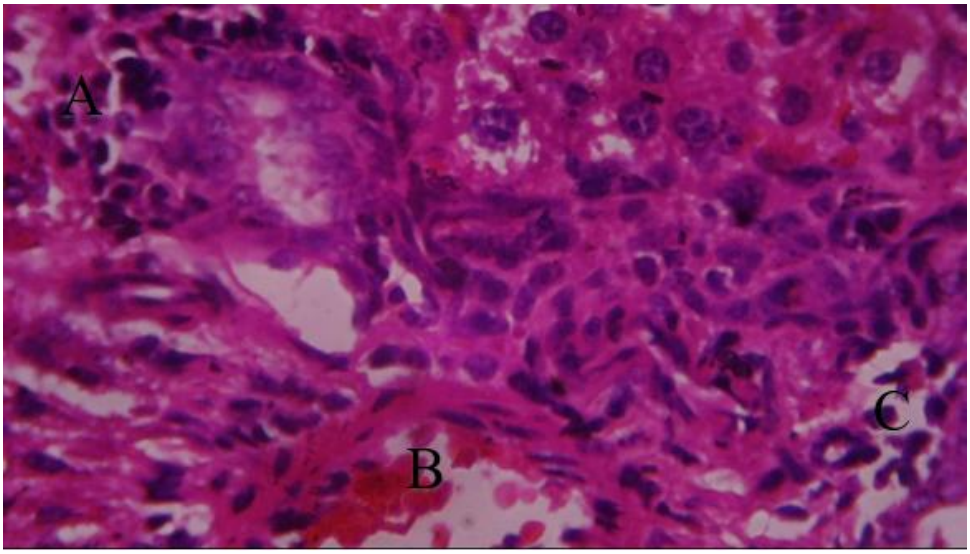

Plate 7: Rat given 400 mg/kg of *L. inermis* + lead acetate showing A: periportal infiltrates of inflammatory cells, B: vascular congestion and C: patchy hepatocyte necrosis (H&E x 400).

DISCUSSION

The preventive and ameliorative effects of *Lawsonia inermis* against lead acetate-induced toxicity in the livers of Wistar rats were investigated in this study. The liver function test data and histopathology were used to assess the toxicity of lead acetate, and the therapeutic properties of Silymarin and *L. inermis*. Silymarin is the most clinically popular medicine for patients with liver injury, and it is recognized to have hepatotherapeutic and anti-fibrotic characteristics (Kim et al., 2007). Silymarin has also been shown to be helpful in a variety of studies, including shielding against genomic harm, enhancing hepatocyte protein synthesis, lowering tumor promoter activity, and stabilizing mast cells (Comoglio et al., 1995; Giacomelli et al., 2002), as a result, it was chosen as the standard drug.

Lead is a very toxic heavy metal with serious public health consequences. Lead poisoning could play a much bigger influence in the development of chronic disorders and impaired functioning than previously thought (Orisakwe, 2014). The administration of lead has been specifically reported to induce liver damage in several reports (Ali et al., 2018; Ghazal and Owolabi, 2012). *Lawsonia inermis* has protected the liver against several hepatotoxins – paracetamol (Selvanayaki and Ananthi, 2012), carbon tetra chloride (Hossain et al., 2011) – where they significantly reduced previously elevated levels of ALP, ALT, AST etc.

Hepatocytes have significant amounts of ALT and AST. When hepatocyte cell membranes and/or hepatocytes themselves are destroyed, these enzymes seep into the bloodstream. Despite this, ALT is thought to be a more specific hepatocellular damage marker than AST because it is only produced in the liver (Kew, 2000), whereas AST is found in a range of tissues including the liver, muscle, and red blood cells (Ramaiah, 2007). It is therefore not surprising that in our study, there was a spike in the levels of liver enzymes in the animals that were administered with lead and also, pathology was observed in their tissues, as seen in Table 2 and Plate 3, respectively, while the pretreatment with *Lawsonia inermis* at the two doses – 200 mg/kg and 400 mg/kg – lowered the levels of the enzymes to amounts comparable with the control group and the standard drug (as seen in Table 2 and Plates 4, 5 and 6). However, L inermis was not so effective as an ameliorative agent, as evidenced by results in Table 2 and Plate 7. This result supports the findings of Bechynska et al (2021) and Kheiripour et al (2019) research, which found Silymarin to be a highly potent hepatoprotective drug.

Hepatocellular dysfunction can be diagnosed by measuring serum bilirubin levels. The increase in serum bilirubin is due to liver injury, as the liver creates excessive amounts of bilirubin as a result of the breakdown of red blood cells and the animals' inability to eliminate bilirubin (Ahmad et al., 2002). After exposure to lead acetate, the amount of bilirubin increased considerably, whereas the aqueous extract of *L. inermis* significantly lowered those levels.

Hepatoprotection can be achieved by either restoring normal hepatic physiology or reducing the toxicant's toxic detrimental effect (Verma et al., 2013). Tannic acid is one of the major constituents of *L. inermis*, according to a study by Ostovari et al (2009). Tannic acid has also been found to have strong anti-oxidant properties (Wu et al., 2004; Andrade et al., 2005). The presence of phenolic compounds in *L. inermis* extract is linked to its hepatoprotective properties (Bhandarkar and Khan, 2003; Jalalpure et al., 2003). The different polyphenolic phytochemicals found in the extract could be responsible for the in vivo protective efficacy of *Lawsonia inermis* aqueous leaf extract against lead acetate-induced liver damage in Wistar rats.

The hepatoprotective and ameliorative activity of *Lawsonia inermis* aqueous leaf extract against lead acetate-induced liver damage in Wistar rats was clearly observed as a result of this investigation. *Lawsonia inermis*' mode of action is yet unknown. The findings imply that *L. inermis* protects against lead acetate-induced hepatotoxicity in a preventive and ameliorating manner. It is, however, more effective when used as a preventative measure.

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