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Original Research Article

Effects of Ethanol Leaf Extract of *Lawsonia inermis* Linn. on Carbon Tetrachloride-Induced Liver Injury In Adult Wistar Rats

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ABSTRACT

Lawsonia inermis L. (Henna) has been reported to possess hepatoprotective, antimicrobial, antioxidant, antifertility, antiulcer, anticancer and immunostimulatory properties. The effects of ethanol leaf extract of *Lawsonia inermis* L. on carbon tetrachloride-induced liver injuries were investigated. Phytochemical screening of extract was conducted using established methods. Forty-five (45) adult Wistar rats weighing between 160g – 185g were randomly assigned into nine (9) groups of five (5) animals each. Groups 1-9 received 1mL of distilled water, 0.5mL/kg of olive oil, 250mg/kg of *L. inermis*, 0.5mL/kg of CCl_4 diluted with olive oil at ratio (1:1), 0.5mL/kg of CCl_4 diluted with olive oil at ratio (1:1) and standard drug Silymarin at dose 100mg/kg, 0.5mL/kg of CCl_4 diluted with olive oil at ratio (1:1) and was then left to recover, 0.5mL/kg of CCl_4 diluted with olive oil at ratio (1:1) and was then treated with 500mg/kg of *L. inermis*, 250mg/kg of *L. inermis* before receiving 0.5mL/kg of CCl_4 diluted with olive oil at ratio (1:1) and 500mg/kg of *L. inermis* before receiving 0.5mL/kg of CCl_4 diluted with olive oil at ratio (1:1) respectively. Administration of ethanol leaf extract of *L. inermis* not only protected the integrity of plasma membrane of hepatocytes, but also increased the regenerative and reparative capacity of the liver, as shown by the significant reductions in the serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and total bilirubin levels. The study demonstrated that the ethanol leaf extract of *Lawsonia inermis* is able to protect and reverse carbon tetrachloride-induced liver injury in Wistar rats.

Keywords: *Lawsonia inermis*, Wistar rats, Carbon tetrachloride, phytochemicals, steatosis.

Introduction

For thousands of years, humans have relied on nature for their essential needs. These needs include food, clothing, shelter, means of transportation, flavours, fragrances and medicines. For thousands of years, plants have formed the basis of sophisticated traditional medicine. One of the earliest records of the use of plants dates back to 2600BC, in Mesopotamia. They used oils of *Cedrus species* (cedar) and *Cupressus sempervirens* (cypress), *Commiphora species* (myrrh) and *Glycyrrhiza glabra* (licorice) to cure illnesses ranging from coughs to colds to parasitic infections and inflammations, and all of these plants still function in present times in the treatment of the aforementioned ailments.[1]

The African continent is made up of more than fifty (50) countries, eight hundred (800) languages and three thousand (3000) dialects, great biodiversity of tropical forests, savannahs and veldts, therefore, it is hardly surprising that it is a veritable treasure house of genetic materials, including medicinal plants. The unique environments of sub-Saharan Africa has presented native cultures with an immense variety of flora and as a consequence, a wealth of traditional knowledge about the use of plants for medicinal purposes.[2]

According to the World Health Organization,[3] as many as 80% of the world's population depend on traditional medicine to meet their primary health care needs and this is due to their accessibility and affordability. In addition, WHO[4] estimated that 80% of inhabitants of developing countries rely on plant drugs as they are natural, innocuous and have no side effects. In Nigeria, traditional medicine has always been the principal form of treatment and presently, there is an upsurge in their popularity, not only in Nigeria, but throughout the world.[5]

Lawsonia inermis (Henna) is a member of the family Lythraceae (also known as the loosestrife family), which is made up of about five hundred (500) species, widely distributed in tropical regions and easily found across the Northern and Southern parts of Nigeria.[6] Henna is cultivated by many farmers for aesthetic and pharmaceutical purposes. The leaves, flowers, seeds, stem bark and roots are all of medical importance as they are used in traditional medicine to treat a variety of ailments. They also act as hepatoprotective agents and as coloring agents.[7]

Lawsonia is named after Sir Isaac Lawson, an 18th century Scottish army doctor. *Inermis* is a Greek word which means "unarmed, without spines" and the description suits the plant .[8] The plant reaches a height of up to 6 meters, and it has scented white or rose-red flowers. Dyes are usually produced from henna plant and they are traditionally drawn on the hands and feet, arms, legs, around the belly button and even behind the neck. The colouring properties of Henna are due to lawsone, a burgundy-coloured organic compound that has an affinity for bonding with protein. Lawsone is largely concentrated in the leaves, especially in the petioles of the leaf.[9]

Quite a number of researchers have reported that Henna plants have been found to exhibit anti-oxidant, anti-diabetic, hepatoprotective, hypoglycemic, antimicrobial and wound healing properties.[9]

The liver is considered the key organ in the metabolism, detoxification and secretory functions in the body and its disorders are numerous with no effective remedies. However, the search for new medicines is still ongoing. Many folk remedies from plant origin have been long used for the treatment of liver diseases. The liver regulates various important metabolic functions and hepatic damage is associated with

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distortion of these metabolic functions. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons many people all over the world, including those in developed countries are turning to complementary and alternative medicine. Many traditional remedies employ herbal drugs for the treatment of liver ailments.[10]

Conventional drugs used in pharmacotherapy such as steroids, vaccines and anti-viral drugs, have shown limited therapeutic benefits and are associated with serious risks of toxicity. In the absence of a reliable hepatoprotective drug in the modern system of medicine, natural extracts from medicinal plants considered to be effective and safe are recommended for the treatment of liver disorders.[11] Silymarin, one of these compounds, is used as a standard reference and exhibits hepatoprotective effects and anti-oxidant activity by altering cytoplasmic membrane architecture and preventing the penetration of hepatotoxic substances, such as carbon tetrachloride (CCl_4), thioacetamide and D-galactosamine.[12, 13]

The reported worldwide ascendancy in liver toxicity as a result of varying factors such as uncontrolled antibiotic use, excessive alcohol intake and the abuse of drugs such as tramadol amongst the youth in Nigeria, has brought about the need for research into a possible solution to the menace. This is especially important because of the complexity of the liver which makes it prone to several ailments and side effects of synthetic drugs such as paracetamol.

Materials and Methods

Identification and collection of plant material

The leaves of *Lawsonia inermis* were obtained from a local market at Egor Local Government Area of Benin City, Edo State. They were subsequently identified at the Herbarium of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria. A sample was deposited at the Herbarium and documented with an identification number – UBHL398. The leaves of the plant were carefully detached and air-dried for seven days. The dried leaves were pulverised into powder form using mechanical grinder. The pulverised plant material was prepared for the ethanol extraction.

Ethanol extraction

One thousand (1000) grams of the powdered leaves of *Lawsonia inermis* was dissolved in five litres of ethanol for seventy-two (72) hours. Thereafter, the preparation was filtered using fine and soft meshwork of iron net to obtain a coloured filtrate. The filtrate obtained was gently heated at regulated temperature (500^oC) to evaporate the solvent using rotary evaporator. The residue obtained was allowed to cool at room temperature and weighed using an electrical weighing balance. The extract obtained was then mixed with ten (10) ml of Tween 80 and dissolved in 490 mL of distilled water to obtain the stock solution of the ethanol extract used for the study. The concentration of the stock solution (in mg/ml) was calculated. The extract was stored in an air-tight container and kept in the refrigerator at 4°C for further uses.

Chemicals

Carbon tetrachloride (CCl_4) used was a product of Abhishek Chemical & Scientific Company, India, while Silymarin was a product of Talent Healthcare, Ahmedabad, Gujarat, India. Both were purchased from local suppliers.

Phytochemical analysis of extract

The extract was subjected to preliminary qualitative analysis for identification of amino acids, alkaloids, naphthoquinones, glycosides, steroidal compounds, saponins and flavonoids, using established methods.[14, 15]

Experimental animals

Forty-five (45) adult Wistar rats of either sex were sourced from the Animal house of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Benin-City, Edo State. They were randomly assigned into nine (9) groups of five (5) animals each. The weight of the experimental animals was between 160 g – 185 g at the onset of the study. Throughout the period of this study, the animals were housed in the Animal House of the Department of Anatomy, University of Benin. Two weeks (14 days) of acclimatization preceded the commencement of the study. All animals received food and water *ad libitum* for the entire duration of the experiment.

The research was carried out in accordance with the guiding principle for the care and use of animals for research committee, which is in line with that set by the World Health Organization [16].

Grouping of animals

Group 1: (Control) received 1 mL distilled water daily by gavage, for a period of thirty-five (35) days.

Group 2: Received 0.5 mL/kg body weight of olive oil (vehicle) by intraperitoneal injection, for seven (7) consecutive days.

Group 3: Received ethanol leaf extract of *L. inermis* (250mg/kg body weight) only, for a period of thirty-five (35) days.

Group 4: Received 0.5 mL/kg body weight of CCl_4 diluted with 0.5 mL/kg body weight of olive oil, by intraperitoneal injection for seven (7) consecutive days.

Group 5: Received 0.5 mL/kg body weight of CCl_4 diluted with 0.5 mL/kg body weight of olive oil, by intraperitoneal injection and 100mg/kg body weight of Silymarin (standard drug) by gavage, for seven (7) consecutive days.

Group 6: Received 0.5 mL/kg body weight of CCl_4 diluted with 0.5 mL/kg body weight of olive oil, by intraperitoneal injection for seven (7) consecutive days and then left to recover for twenty-eight (28) days.

Group 7: Received 0.5 mL/kg body weight of CCl_4 diluted with 0.5 mL/kg body weight of olive oil, by intraperitoneal injection for seven (7) consecutive days and was afterwards treated with ethanol leaf extract of *L. inermis* (500mg/kg body weight) for twenty-eight (28) consecutive days.

Groups 8: Received ethanol leaf extract of *L. inermis* (250 mg/kg body weight) for twenty-eight (28) days consecutively, and subsequently received of 0.5 mL/kg body weight of CCl_4 diluted with 0.5 mL/kg body weight of olive oil, by intraperitoneal injection daily for seven (7) consecutive days.

Group 9: Received ethanol leaf extract of *L. inermis* (500 mg/kg body weight) for twenty-eight (28) days consecutively and afterwards received of 0.5 mL/kg body weight of CCl_4 diluted with 0.5 mL/kg body weight of olive oil, by intraperitoneal injection daily for seven (7) consecutive days.

Induction of hepatotoxicity

The 7-day intraperitoneal injection of 0.5ml/kg bw of CCl_4 dissolved in olive oil at a ratio 1:1 induced liver damage, according to established methods.[17-19]

Collection of tissue samples

After administration, the Wistar rats in all groups were weighed to determine their final weights. They were anaesthetized with chloroform and sacrificed. Blood was collected by cardiac puncture using a syringe and needle into plain bottles. The livers were excised, weighed and placed in universal bottles containing 10% neutral buffered formalin solution prior to tissue processing.

Biochemical assays

The separated serum was analyzed for various liver enzymes activities. These include the levels of serum alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase and total bilirubin concentration using appropriate methods and kits.

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Tissue processing
At the end of the study period, the harvested liver tissues of all experimental animals were subjected to established processing procedures to preserve and convert them into forms or sections that can be viewed and analyzed under the microscope.[20]

Photomicrography
Stained slides were viewed using an optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany) and photomicrographs were taken at x100 magnification using an attached Eakins 14MP digital microscopic camera.

Measurements
The Wistar rats in all groups were weighed pre-test and post-test. The changes in the body weight across all groups were measured for weight loss and weight gain. The liver was also weighed at sacrifice to ascertain the hepatosomatic index of each animal.

Statistical analysis
All data were subjected to statistical analysis using the IBM SPSS statistics software (Statistical Package for Social Science) (Version 25) and relevant statistical values were obtained. The values of the treated groups were compared with those of non-treated group using the one-way analysis of variance (ANOVA) and the t-test method. Values of $P < 0.05$ were considered significant. The statistical values obtained were converted into graphical representations in the form of bar charts.

Results and Discussion

Phytochemical analysis
Table 1 show that the ethanol leaf extract of *Lawsonia inermis* contains steroidal compounds, flavonoids, naphthoquinones and tannins. However, saponins, alkaloids and amino acids were absent.

Hepatic weight
Results obtained from weighing the livers (Figure 1) showed a significant increase ($P < 0.05$) in hepatic weight in Groups 4 and 6, when compared to the control group. The other treatment groups were comparable with the control group.

Change in body weight
Results obtained from the analysis of the initial and final body weights of the experimental animals (Figure 2) revealed a significant increase ($P < 0.05$) of final body weight in Groups 1, 3, 5 and 9, when compared to the initial body weight. However, there was a significant decrease ($P < 0.05$) in final body weight in Group 4, when compared to the initial body weight.

Hepatosomatic index (HI)
Results obtained from calculating the hepatosomatic index across the experimental groups (Figure 3) showed a significant increase ($P < 0.05$) in hepatosomatic index in Groups 4 and 6, when compared to the control group. The HI of the other treatment groups were comparable to the control group.

Biochemical assays
Aspartate aminotransferase: There was a significant increase ($P<0.05$) in serum AST levels in Groups 4 and 6, when compared to the control group. The serum AST levels of other treatment groups were comparable to that of the control group (Figure 4).

Alanine aminotransferase: There was a statistically significant increase ($P<0.05$) in serum ALT levels in Groups 4, 6 and 7, when compared to that of the control group. The serum ALT levels of the other treatment groups were comparable to that of the control group (Figure 5).

Alkaline phosphatase: There was a statistically significant increase ($P<0.05$) in serum ALP levels in Groups 4, 6 and 7, when compared to that of the control group. The serum ALP levels of the other treatment groups were comparable to that of the control group (Figure 6).

Table 1: Phytochemical constituents of ethanol leaf extract of *Lawsonia inermis* L.

Phytochemical	Inference
Alkaloids	-
Amino acids	-
Steroidal compounds	+
Saponins	-
Flavonoids	+
Naphthoquinones	+
Tannins	+

Total bilirubin: There was a statistically significant increase ($P<0.05$) in total bilirubin levels in Groups 4 and 6, when compared to that of the control group. The levels of the other treatment groups were comparable to that of the control group (Figure 7).

Histology of the liver
Plate 1 is a photomicrograph showing the liver of the Wistar rat of Group 1 (control group) which received 1 mL distilled water daily by gavage, for a period of thirty-five (35) days. It shows a normal histoarchitecture comprising central vein, radiating plates of hepatocytes and sinusoids. Plate 2 is a photomicrograph of Group 2 which received 0.5ml/kg body weight of olive oil (vehicle) by intraperitoneal injection, for seven (7) consecutive days. It also shows a normal histoarchitecture.
Plate 3 is a photomicrograph of Group 3 depicting the liver administered with ethanol leaf extract of *L. inermis* (250 mg/kg body weight) only, for a period of thirty-five (35) days. It also shows a normal histoarchitecture. Plate 4 is a photomicrograph of Group 4 which received 0.5 mL/kg body weight of CCl_4 diluted with 0.5 mL/kg body weight of olive oil, by intraperitoneal injection for seven (7) consecutive days. It reveals severe intacytoplasmic hepatocyte fat vacuolation (steatosis) and portal congestion.
Plate 5 is a photomicrograph of Group 5 which received 0.5 mL/kg body weight of CCl_4 diluted with 0.5 mL/kg body weight of olive oil, by intraperitoneal injection and 100 mg/kg body weight of Silymarin (standard drug) by gavage, for seven (7) consecutive days. It shows a normal histoarchitecture. Plate 6 is a photomicrograph of Group 6 which received 0.5 mL/kg body weight of CCl_4 diluted with 0.5 mL/kg body weight of olive oil, by intraperitoneal injection for seven (7) consecutive days and then left to recover for twenty-eight (28) days. It shows moderate intracytoplasmic hepatocyte fat vacuolation (steatosis), moderate vascular congestion, and heavy periportal infiltrates of inflammatory cells.
Plate 7 is a photomicrograph of Group 7 which received 0.5ml/kg body weight of CCl_4 diluted with 0.5ml/kg body weight of olive oil, by intraperitoneal injection for seven (7) consecutive days and was afterwards treated with ethanol leaf extract of *L. inermis* (500 mg/kg body weight) for twenty-eight (28) consecutive days. It shows vascular congestion and moderate steatosis. Plate 8 is a photomicrograph of Group 8 which received low dose of ethanol leaf extract of *L. inermis* (250mg/kg body weight) for twenty-eight (28) days consecutively, and subsequently received of 0.5 mL/kg body weight of CCl_4 diluted with 0.5 mL/kg body weight of olive oil, by intraperitoneal injection for seven (7) consecutive day. It shows lymphocytes mobilised around the portal zone, central vein, mobilization of moderate population of sinusoidal Kupffer cells and normal hepatocytes.

Plate 9 is a photomicrograph of Group 9 which received ethanol leaf extract of *L. inermis* (500 mg/kg body weight) for twenty-eight (28) days consecutively and afterwards received of 0.5 mL/kg body weight

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of CCl_4 diluted with 0.5ml/kg body weight of olive oil, by intraperitoneal injection daily for seven (7) consecutive days. It shows normal portal vein and small intracytoplasmic hepatocyte fat vacuolation.

Reactive oxygen species (ROS) from both endogenous and exogenous sources, may be involved in the etiologies of such diverse human diseases as arteriosclerosis, ischemic injury, cancer, and neurodegenerative diseases, as well as in processes like inflammation and ageing.[21, 22] There is evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress. There is also increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs, and medicinal plants.[23, 24]

Carbon tetrachloride (CCl_4) is a xenobiotic industrial solvent that is used to induce chemical hepatitis and liver injuries in experimental animals. Carbon tetrachloride-induced liver injuries are the most common experimental models for monitoring the hepatoprotective activity of certain drugs. A single exposure to CCl_4, as being a strong hepatotoxic xenobiotic, directly leads to severe liver necrosis and steatosis.[25] The changes associated with CCl_4-induced liver damage are comparable to that of acute viral hepatitis.[26, 27]

Usually, the therapeutic value of medicinal plants may be accessed through determination of the biological activity of their phytochemical constituents. The result of the preliminary phytochemical profiling of the ethanol leaf extract of *L. inermis* (Table 1) revealed the presence of compounds with diverse biological activities and pharmacological relevance. The compounds present are steroidal compounds, flavonoids, naphthoquinones and phenolic compounds. The results are in agreement with Raja *et al*,[28] who carried out a similar study. However, alkaloids, amino acids and saponins were absent. This is also consistent with the findings by Jain *et al*.[29]

Flavonoids are a large group of naturally occurring phenolic compounds pervasively distributed in the plant kingdom.[30] Acacetine, also known as linarigenin or linarisenin, luteolin [31], apiin, cosmosiin,[32] isoscutellarin,[33] rhoifolin,[34] apigenin and genistein [35] are all flavonoids that have been isolated from the aerial parts and leaves of *Lawsonia inermis.* In addition to direct or indirect inhibition of oxidases, the anti-oxidant properties of flavonoids are due to their ability to scavenge free radicals, to react with non-radicalic reactive oxygen species. Hepatoprotective properties of flavonoids have also been reported in numerous experimental *in vivo* studies. In some of these reports, there was a general consensus that the antioxidative properties of the flavonoids (most importantly) participated in their hepatoprotective activities. For example, in rats fed an iron-enriched diet for four (4) months, silibinin (a natural flavonoid) largely decreased lipid peroxidation and liver injury.[36]

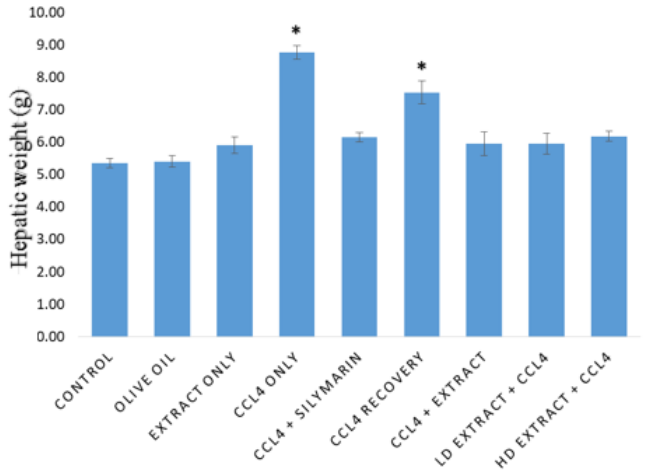

Figure 1: Hepatic weight across all groups. LD Extract = Low dose extract, HD Extract = High dose extract. *significantly different from the control group.

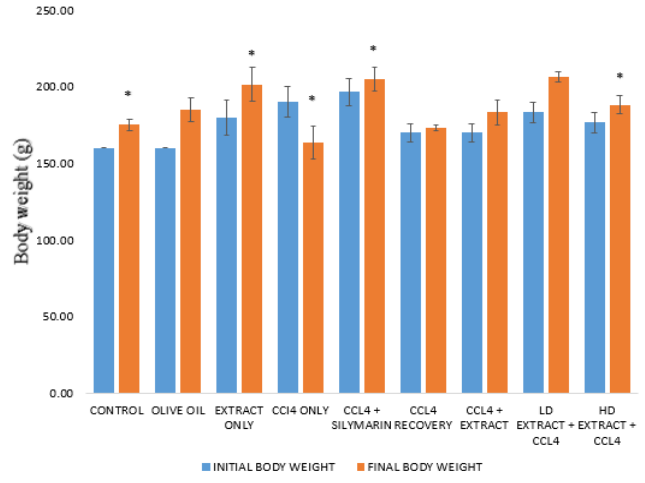

Figure 2: Comparison between initial body weight and final body weight across all groups. LD Extract = Low dose extract, HD Extract = High dose extract. *significantly different from the initial body weight.

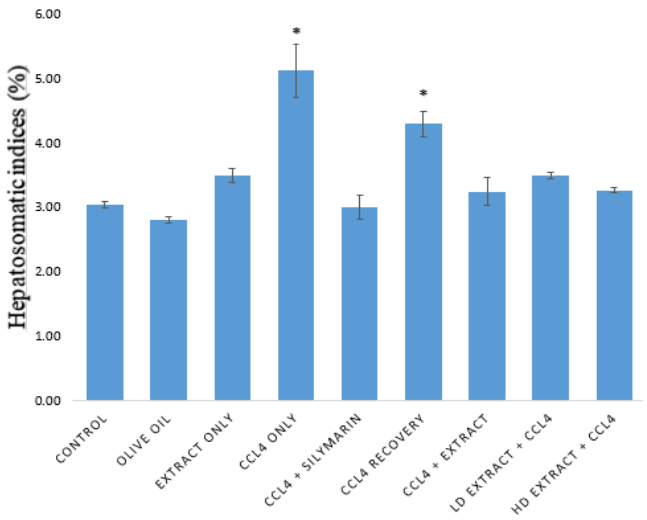

Figure 3: Hepatosomatic indices across all groups. LD Extract = Low dose extract, HD Extract = High dose extract. *significantly different from the control group.

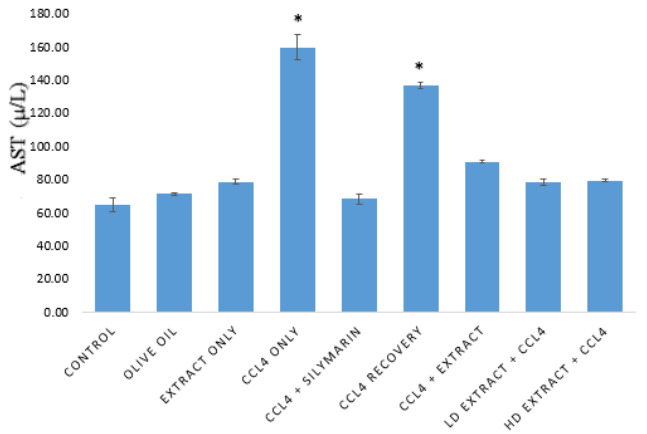

Figure 4: Levels of serum AST across the groups. LD Extract = Low dose extract, HD Extract = High dose extract. *significantly different from the control group.

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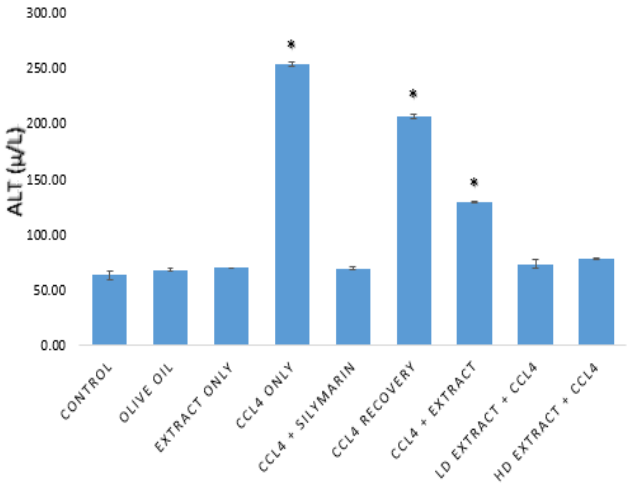

Figure 5: Levels of serum ALT across the groups. LD Extract = Low dose extract, HD Extract = High dose extract. *significantly different from the control group.

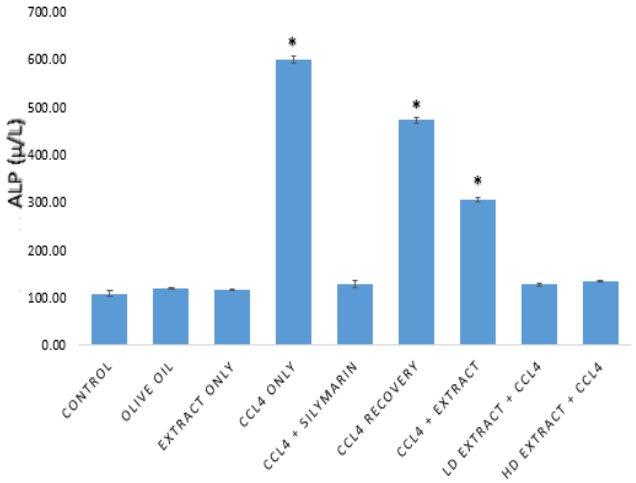

Figure 6: Levels of serum ALP across the groups. LD Extract = Low dose extract, HD Extract = High dose extract. *significantly different from the control group.

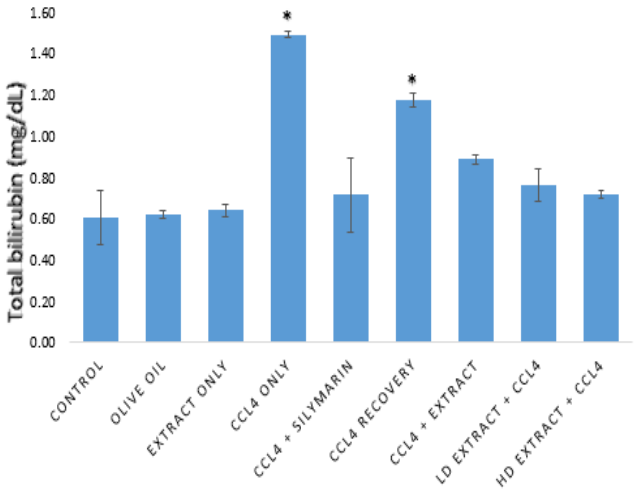

Figure 7: Levels of total bilirubin across the groups. LD Extract = Low dose extract, HD Extract = High dose extract. *significantly different from the control group.

A study by Ostovari *et al* [37] revealed that tannic acid is one of the main constituents of *L. inermis.* Tannic acid has also been shown to possess impressive anti-oxidant activity.[38 - 40] The hepatoprotective effects of henna extracts are associated with the presence of phenolic compounds. Polar extracts have been proven to have a protective effect against CCl_4-induced liver toxicity in mice and rats.[41, 42]

According to the results of this study, the presence of phytochemical compounds with antioxidant activities in the ethanol leaf extracts of *L. inermis* highlights their hepatoprotective effect through significant reduction in the level and activities of free radicals in the hepatic tissue. Similar conclusions that related hepatoprotection and anti-oxidant activities were also made by Park *et al* [43] and Nanji *et al.*[44] It has been established that the target organ for carbon tetrachloride (CCl_4) is the liver, and that the administration of CCl_4 leads to numerous changes in the liver. Amongst the changes, CCl_4 has been reported to cause an increase in liver weight.[45, 46] Uemitsu and Nakayoshi [47] also concluded that CCl_4 does induce an increase in liver weight following a single dose. This phenomenon was also reported in this current study, as shown in Figure 1, where the group administered with CCl_4 only (Group 4) and the CCl_4 recovery group (Group 6) showed a significant increase ($P < 0.05$) in hepatic weight. Results obtained from Group 4 and Group 6 were suggestive of hepatomegaly. Hepatomegaly is a term used to describe a liver that is enlarged beyond its normal size. In itself, hepatomegaly is not a disease, but rather an indicator of a potential underlying disease process.[48] This increase in liver weight may be attributed to the fatty ingrowth (steatosis) observed in this study. Similar findings have been reported in several studies after the administration of CCl_4.[49, 50] However, the administration of *L. inermis* prevented and protected the liver from such changes, as shown in Figure 1.

The administration of CCl_4 also caused a significant increase in hepatosomatic index. Hepatosomatic index (HSI) is the weight of the liver expressed as a percentage of total body weight (also known as liversomatic index).[51] This change was also nullified by the administration of *L. inermis.*

That there was no significant change in the weight of the livers of Group 3 which received only *L. inermis* may imply that no damage occurred. This is in line with the findings of Chikaraddy *et al* [52] which showed that the plant extract is safe up to a dose of 2000mg/kg body weight. During the duration of the experiment, Groups 4 and 6 showed a noticeable decrease in the amount of feed intake (inappetence). A similar finding was reported by Uemitsu and Nakayoshi [47]. In addition to a loss of appetite, Groups 4 and 6 also exhibited delayed responsiveness and a reduction in locomotory activities.

Among the many drugs for liver injury, silymarin is the most clinically popular for patients and is known to have hepatotherapeutic and anti-fibrotic properties.[53] Silymarin has also been proven effective in several research fields, such as protecting against genomic injury, increasing hepatocyte protein synthesis, decreasing the activity of tumor promoters and stabilizing mast cells. According to many authors however, it has a low bioavailability.[54, 55]

In this study, the hepatotoxin (CCl_4) was responsible for the unsubtle damage to the liver, as revealed by the elevation of serum AST, ALT and ALP activities. An upsurge in the levels of total bilirubin was also recorded. Serum aminotransferase concentration is an indicator of hepatocellular damage. ALT and AST are available in high concentrations in hepatocytes. However, when the cell membranes of hepatocytes and/or the hepatocytes themselves are damaged, these enzymes leak into the circulation. In spite of this, ALT is considered to be the more specific hepatocellular injury marker because it is found exclusively in the liver,[56] compared to AST, which is present in a variety of tissues such as liver, muscle and red blood cells.[57] Although AST and ALT activities are sensitive indicators of liver-cell damage, neither alone is an ideal marker for hepatocellular damage.[56] Nevertheless, serum levels of AST and ALT rise and fall in parallel [58].

In the plasma membrane of hepatocytes, ALP is present. An upsurge in its level is linked to damage of the liver's cell membrane.[59] Results indicated in Figure 6 show a significant increase of ALP in the CCl_4-administered group when juxtaposed with the control group.

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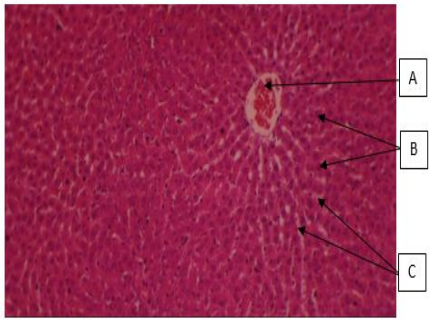

Plate 1: Control rat showing normal liver composed of A: central vein, with B: radiating plates of hepatocytes, separated by C: sinusoids (H&E x 100)

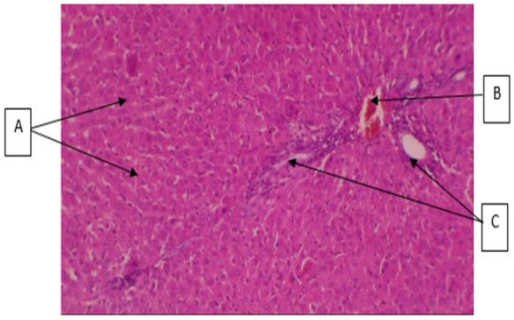

Plate 1: Rat liver given olive oil only, showing A: normal hepatocytes, B: portal vein and C: bile ducts (H&E x 100)

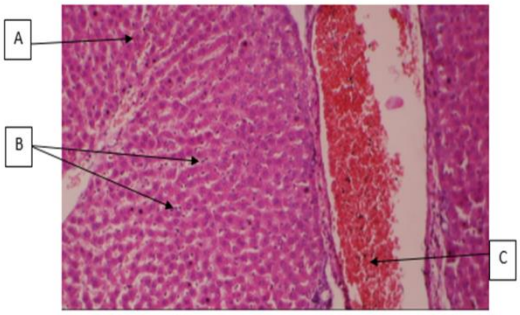

Plate 2: Rat liver given extract only, showing A: normal hepatocytes, B: activated sinusoidal Kupffer cells and C: dilated portal vein with active congestion (H&E x 100)

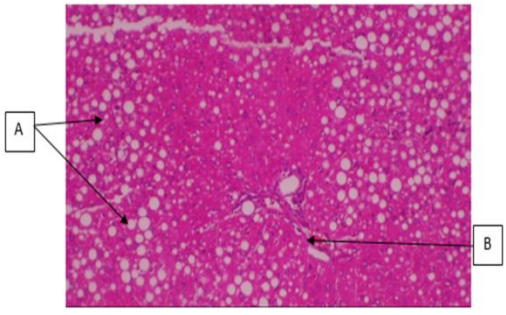

Plate 3: Rat liver given CCl_4, showing: A: mixed vesicular fat accumulation (steatosis) and B: portal congestion (H&E x 100)

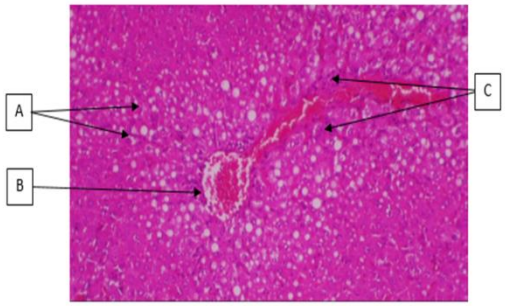

Plate 4: Rat liver intoxicated for 7 days and left to recover for 28 days, showing A: mixed vesicular fat accumulation (steatosis), B: moderate vascular congestion and C: periportal infiltrates of inflammatory cells (H&E x 100)

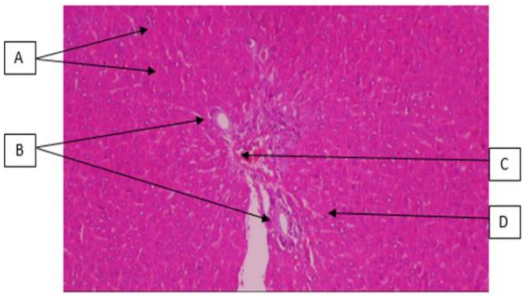

Plate 5: Rat liver given Silymarin + CCl_4 showing A: normal hepatocytes, B: bile ducts, C: portal vein and D: mild Kupffer cell activation (H&E x 100)

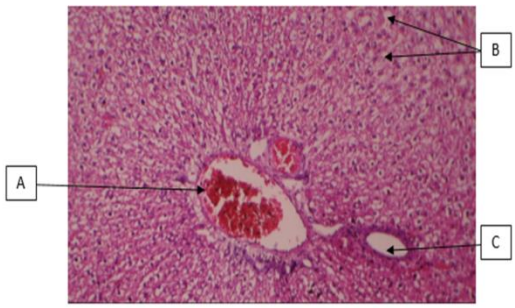

Plate 6: Rat liver assaulted with CCl_4 for 7 days, and thereafter treated with high dose extract, showing A: vascular congestion, B: patchy areas of steatosis and C: normal bile duct (H&E x 100)

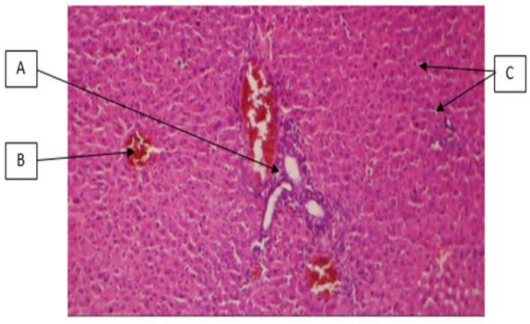

Plate 7: Rat liver treated with low dose extract for 28 days and thereafter, assaulted with CCl_4 for 7 days showing A: mobilization of lymphocytes around portal triad, B:active vascular congestion, and C:mobilization of sinusoidal Kupffer cells (H&E x 100)

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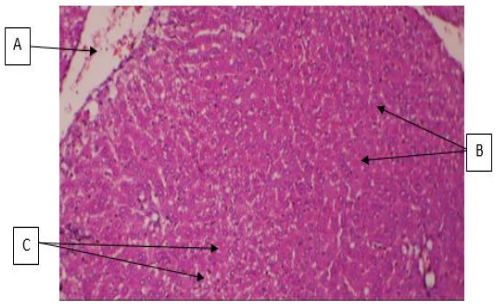

Plate 8: Rat liver treated with high dose extract for 28 days and thereafter, assaulted with CCl4 for 7 days showing A: mild portal vein dilatation, B: normal hepatocytes and C: patchy intracytoplasmic hepatocyte fat vacuolation (H&E x 100)

Normal values of ALP in Wistar rats of the control group were found to be 108.22 ± 5.04 which rose significantly ($P < 0.001$) to 600.64±7.76 after the administration of toxic doses of CCl_4. The pretreatment of the experimental animals with the extract in low dose (250 mg/kg body weight) and high dose (500mg/kg body weight) in groups 8 and 9 respectively also showed a significant reduction in the ALP level, thereby, substantiating its reported hepatoprotective properties. The extract also halted the increase in ALP levels in Group 7, albeit, insignificantly ($P > 0.05$) when compared with the control group, while the group that received the extract only showed comparable values with the control group, maintaining its reported safety.

Steatosis or fatty change of the liver is the accumulation of abnormal amounts of lipids in 5% or more hepatic cells. Macrovesicular steatosis is common. It is frequently made apparent by noninvasive imaging and may be accompanied by moderate abnormalities of serum aminotransferases, alkaline phosphatase, and gamma glutamyl transpeptidase.[60]

Histologically, steatosis is physically apparent as lipid within membrane bound liposomes of parenchymal cells. When the liver tissue is fixed and stained to be better viewed under a microscope, the lipid is usually dissolved by the solvents used to prepare the sample. As such, samples prepared this way will appear to have empty holes (or vacuoles) within the cells where the lipid has been cleared.[61]

From the result of this study, the hepatoprotective and ameliorative potency of ethanol leaf extracts of *Lawsonia inermis* L. was prominently observed. The probable mechanism of action of *Lawsonia inermis* appears to be its effect as a free radical scavenger and inhibitor of lipid peroxidation of the liver plasma membrane. The results suggest that *L. inermis* is both proactive and responsive in protecting against CCl_4-induced hepatotoxicity. However, it is more bioactive when administered prophylactically.

Conclusion

The effects of ethanol leaf extract of *Lawsonia inermis* L., like other medicinal plants, are determined by different variables, but essentially assessed by their component phytochemical constituents. The contents of the extract not only protected the integrity of plasma membrane but, at the same time, increased the regenerative and reparative capacity of the liver. This study has demonstrated that the ethanol leaf extract of *Lawsonia inermis* is able to protect or reverse carbon tetrachloride-induced liver injury in Wistar rats. Our results further support the view that some traditionally medicinal plants (for example, *Lawsonia inermis*) are promising sources of potential antioxidants.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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