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Research Article

Investigating the Protective Effect of *Lawsonia inermis* Extract on Liver and Kidney Function in Carbon Tetrachloride (CCl_4) Induced Rats

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Abstract. A lasting cure for liver and kidney damage caused by exposure to toxic substances has continued to elude contemporary medicine. This has resulted in an ever increasing dependence on alternative and traditional herbal medicine as a means of management of liver and kidney toxicities. The present study evaluates the protective effect of *Lawsonia inermis* leaves extract on carbon tetrachloride (CCl_4) induced toxicity in wistar albino rats. Animals were grouped into five (5) with Group I (distilled water) serving as control. Group III was pretreated with silymarin; Groups IV and V received 100mg/kg and 200mg/kg of *Lawsonia inermis* respectively. Groups III – V were administered CCl_4 on the 7th day of the experiment. Serum was collected from the animals 24 hours after induction and on the 14th day of the experiment. Extracts of *Lawsonia inermis* leaves was observed to show protective effect by lowering CCl_4 elevated serum enzyme markers like alanine and aspartate aminotransferases (ALT and AST); lipid profiles such as Total cholesterol (TC), triacylglycerols (TAG), low density lipoprotein (LDL-c); bilirubin, urea and creatinine while concomitantly increasing high density lipoprotein (HDL-c), total protein and albumin. Extract initiated reversals were found to be dose dependent and significant ($P < 0.05$). The findings in the present study indicates that pretreatment with *Lawsonia inermis* showed protective effect in vivo in CCl_4 compromised liver and kidneys.

Keywords: *Lawsonia inermis*, toxicity, serum, lipid profile, creatinine, liver, Kidney, CCl_4

1. Introduction

Environmental pollution, occupation, lifestyle and diet are among some of the major predisposing factors that expose humans and animals to several chemical agents. Most of these chemical agents or substances tend to become toxic and cause harm when bio-transformed within the body. Toxicity depend on a variety of factors which include; dose, duration, route of exposure, shape and structure of the chemical itself, and the individual human factors [1, 2].

The liver is the largest internal organ and serves as the principal organ for maintaining the body's internal environment [3, 4]. It plays a myriad of vital roles in the maintenance, performance and regulation of homeostasis. It is involved with almost all the biochemical pathways necessary for growth and development, fight against disease, supply of nutrient, providing energy, secretory and excretory functions, as well as reproduction [5, 6]. Exposure to high levels of xenobiotics poses a threat of injury and normal function of the liver [5].

The kidneys play an important role in the filtration and secretion of the end products of metabolism and excess of electrolytes [7]. They also play key roles in the maintenance and regulation of homeostasis [8]. Due to its unique role in metabolism and close relationship with the gastrointestinal tract the kidney is susceptible to chemical induced injury [9, 10]. Nephrotoxicity results in serious clinical syndromes, including Acute Kidney Injury (AKI) caused by industrial pollution, occupational hazard, alcoholism and substance abuse [11].

Carbon tetrachloride (CCl_4) is a clear, colorless, and volatile hydrocarbon which was previously used in some dry-cleaning agents, refrigerants and pesticides. It has been found to be a human carcinogen and also a hepatic and renal toxicant [12, 13]. In the body, CCl_4 is activated by cytochrome P450 (CYP2E1, 2B1 or 2B2), to form the trichloromethyl radical (CCl_3*) or trichloromethylperoxy radical (CCl_3OO*), which binds to bio-molecules and impair crucial cellular processes such as lipid metabolism culminating in fatty degeneration [14]. This may affect plasma, mitochondrial and endoplasmic membrane permeability, resulting in the loss of cellular calcium sequestration and homeostasis, which can contribute heavily to subsequent cell damage [15]. Elevations in serum enzyme levels are taken as the relevant indicators of liver toxicity whereas increases in both total and conjugated bilirubin levels are measures of overall liver function [16]. Carbon tetrachloride induced toxicity is irreversible, with treatment only seen to minimize the effects of the toxin and ease symptoms.

There is an ever increasing number of the world population who depend on traditional herbal medicine for treatment and management of diseases with an estimated 80% of the world population depending on plant based remedies for primary healthcare. There are a number of traditional treatments, involving either a plant extract or a mixture of plant extracts found to have therapeutic effects on toxicity [17]. Medicinal plants play an important role in the maintenance of health and vitality, but also in the cure of several diseases, including liver disorders without causing any toxicity. Medicinal plants contain several phytochemicals, which possess strong antioxidant activities [18, 19]. These antioxidant phytochemicals may include flavonoids, terpenoids, polyphenols, alkaloids, saponins, vitamins, carotenoids, minerals [18]. Some plants used in traditional folk medicine for the treatment of toxicity are discussed by Pandey [20].

Lawsonia inermis (Henna), a member of the Lythraceae, is cultivated in many regions as an ornamental and commercial dye crop. It is widely distributed across tropical and semi-arid regions of Africa and Asia [21]. Varghese *et al*., [22], reported on a variety of secondary metabolites which have been isolated from Henna. These include; naphthoquinone derivatives, phenolics, coumarins, xanthones, tannins, flavonoids, triterpenes, and sterols.

L. inermis has been used utilized in folklore medicine as an astringent, hypotensive, sedative, and against headaches, leprosy, and jaundice [23, 24]. Leaves have also been used for skin and venereal diseases, smallpox, bronchitis, scabies, boils, and sores [25]. Powdered seeds were effective against dysentery and liver disorders. The bark is used in a variety conditions, such as burns, jaundice, spleen enlargement, leprosy, and skin disorders. Roots of *L. inermis* were considered as a potent medicine for some sexually transmitted infections such as; gonorrhea and herpes [26].

Despite the increasing burden of liver and kidney diseases, available treatment options are problematic. The effectiveness of orthodox medicines is inconsistent and is often accompanied by debilitating adverse reactions [27]. This study seeks to investigate the effect of ethanol extract of *L. inermis* on some liver and kidney parameters in acute and sub acute CCl_4 induced toxicity in wistar albino rats.

2. Materials and Methods

2.1. Drugs and chemicals. ALT, AST, HDL, Triglycerides, Cholesterol, Albumin, Total Protein Urea (Randox Laboratories, United Kingdom), creatinine (Agape Diagnostics, Switzerland), Ethanol (BHD England), Carbon tetrachloride (Kermel analytical reagent, China), were all obtained from the respective sources in parenthesis. Silymarin (Micro Labs Limited, India) standard drug was obtained from Vincar pharmacy. All other reagents used in the course of the experiment were of analytical grade.

2.2. Preparation of plant material. Freshly collected leaves of *L. inermis* (Henna) were identified and authenticated by Mr. Ojobo O. of the Department of Botany, Federal University of Agriculture, Makurdi. Leaves were then washed with water to ensure the removal of adhering dirt and subsequently air dried. The dried leaves were then pulverized to coarse powder with the aid of a grinding machine and sieved. About seventy five grams (75g) of pulverized plant material was macerated in 750ml of 99% ethanol for 72 hours at room temperature. The extract was filtered using Whatmann's filter paper number 1, with the Filtrate evaporated using a water bath and stored in a refrigerator at 4°C prior to use.

2.3. Experimental animals. Healthy male albino rats of Wistar strain (~180g – 230g), purchased from the animal house of the college of Veterinary Medicine, University of Agriculture, Makurdi were housed in well ventilated cages under conditions of 12 hour light-dark cycle and temperature of 25°C. Animals were allowed to acclimatize for a period of two weeks and fed with a standard balanced commercial pellet diet (Hi- Breed Feeds Ltd. Benue state, Nigeria) and potable tap water. All experiments were carried out in compliance with the guidelines and recommendations of the ethics committee on the care and use of laboratory animals of the University of Agriculture (UAM/ECA/10208/18), Makurdi.

2.4. Acute toxicity studies. Acute toxicity study of the leaf extract of *L. inermis* was carried out according to the method described by Lorke [28].

2.5. Experimental design. Twenty-five (25) albino rats were used in this experiment. Animals were randomly divided into five (5) groups of 5. Administration of plant extracts and Silymarin was done via oral intubation. Administration of CCl_4 was done by intraperitoneal injection. All groups with the exception of Group I received CCl_4 on the 7th day. The treatment groups are described as follows:

Group I: received normal saline

Group II: received 2ml/kg b.wt of CCl_4 on the 7th day

Group III: received 100mg/kg body weight Silymarin.

Group IV: received 200mg/kg body weight *L.inermis* leaves extract.

Group V: received 400mg/kg body weight of *L.inermis* leaves extract.

Administration of ethanol leaves extracts of *L. inermis* and Silymarin were were carried out daily for the duration of the experiment. The animals were sacrificed under mild Ketamine anaesthesia in two batches; first, 24 hours and, 7 days after induction with CCl_4.

2.6. Blood sample collection. Blood samples were collected from the animals by means of cardiac puncture under ketamine anaesthesia. Samples were obtained from animals on the 8th and 14th day of the experiment, in plain sterile test tubes, followed by centrifuging at 3000rpm x 10min using a Uniscope centrifuge and subsequent separation of the blood plasma.

2.7. Serum determinations

2.7.1. Determination of total protein, albumin and total bilirubin. Total protein determination was carried out by the method of Tietz [29]; serum Albumin by the method described by Doumas *et al*., [30]; and serum total bilirubin by the method described by Jen-drassik and Grof [31].

2.7.2. Determination of liver enzymes. Serum AST and ALT were determined using the method of Reitman and Frankel [32].

2.7.3. Determination of lipid profiles. Serum lipid profiles were determined using the methods described by the investigators in parenthesis. Total cholesterol, (Allain, 1974); Triacylglycerols, (McGowan, 1983); HDL-c, (Freidwald *et al*., 1972); and LDL-c, (Bauer, 1982).

2.7.4. Determination of creatinine and urea. Serum urea was determined by the method described by Searcy [37], while Creatinine was determined by the method described by Bartels and Bohmer [38].

2.8. Statistical analysis. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's multiple Range Test (DMRT). Values are expressed as means ± S.E. of mean and considered to be significant at a value of $P < 0.05$.

3. Results

3.1. Acute toxicity test. Assessment of acute lethal dose (LD_{50}) of *L. inermis* leaves extract in wistar albino rats revealed that none of the animals exhibited signs of toxicity and no mortality was recorded. Therefore, the oral median lethal dose (LD_{50}) was estimated to be more than 5000mg/kg body weight.

3.2. Effect of extract on Serum Total protein, Albumin and Bilirubin. Table 1 shows results of the effect of *L. inermis* on serum bilirubin, albumin and total protein in CCl_4 induced toxicity. Serum total protein and albumin levels were significantly decreased ($P < 0.05$) in groups receiving CCl_4 (Group II) when compared with animals receiving only distilled water (Group I). Treatment with ethanol extracts of *L. inermis* leaves however, resulted in increases which were significant ($P < 0.05$) on day 14 of the experiment. Serum total biliurbin levels were significantly ($P < 0.05$) elevated on induction with CCl_4, conversely, pre-treatment with plant extracts show a reduction in total bilirubin levels on day 14 of the experiment.

3.3. Effect of extract on serum Aspartate aminotransfarase and Alanine amino transfarase. The result for serum AST and ALT activities obtained following CCl_4 induced toxicity revealed significant increases ($P < 0.05$) on day 8. Pre-treatment with leaves extract, however, led to significant ($P < 0.05$) decreases in the serum AST and ALT activities on day 14. Extract showed dose dependent reduction of serum enzyme levels. The results of the experiment are displayed on table 2.

3.4. Effect of extract on serum lipid profile. Results for the evaluation of the effect of extract on serum lipid profile are displayed in Table 3. Induction with CCl_4 showed significant ($P < 0.05$) increase in serum levels of total cholesterol, TAG, and LDL-c and simultaneous decrease in HDDL-c. However, pre-treatment with extracts induced significant ($P < 0.05$) reduction in serum total cholesterol, TAG and LDL-c and an increase in HDL-c levels on day 14 of the experiment. Reversals of the effect of extracts on CCl_4 induced toxicity were also found to be dose dependent for lipid parameters tested.

3.5. Effect of extract on serum Urea and Creatinine levels. Results obtained for experiment following CCl_4 induced toxicity reveals significant ($P < 0.05$) elevation in serum urea

Table 1: Effect of extract on Total Protein, Albumin and Bilirubin.

Treatment	T. Prot 24hrs (g/dl)	T. Prot day 14 (g/dl)	Albumin 24hrs (g/dl)	Albumin day 14 (g/dl)	T. Bil day 14 (g/dl)
GROUP I	7.54 ± 0.42^{a}	8.37 ± 0.57^{a}	2.30 ± 0.08^{a}	3.79 ± 0.07^{a}	1.13 ± 0.06^{a}
GROUP II	5.91 ± 0.19^{b}	6.14 ± 0.22^{b}	1.73 ± 0.23^{b}	2.17 ± 0.04^{b}	1.54 ± 0.14^{b}
GROUP III	6.45 ± 0.35^{ab}	7.59 ± 0.44^{ab}	2.17 ± 0.07^{a}	3.59 ± 0.06^{c}	1.19 ± 0.08^{ab}
GROUP IV	6.34 ± 0.35^{b}	7.34 ± 0.34^{ab}	1.99 ± 0.11^{ab}	3.31 ± 0.03^{c}	1.50 ± 0.14^{ab}
GROUP V	6.34 ± 0.63^{b}	7.64 ± 0.71^{ab}	2.10 ± 0.02^{ab}	3.33 ± 0.05^{c}	1.19 ± 0.15^{ab}

Key: Data are expressed as means ± SEM (n = 5). Values with different alphabets within a column are statistically significant at $P < 0.05$. Group I- Normal control, Group II- CCl_4 induced control, Group III- Silymarin, Group IV- 200mg/kg b.wt of *L.inermis* + CCl_4 Group V- 400mg/kg b.wt of *L. inermis* + CCl_4.

Table 2: Effect of extract on Liver indices.

Treatment	AST 24hrs(U/l)	AST day 24(U/l)	ALT 24hrs (U/l)	ALT day 24 (U/l)
GROUP I	59.11 ± 10.03^{a}	79.77 ± 8.93^{a}	24.85 ± 0.28^{a}	28.17 ± 0.48^{a}
GROUP II	115.18 ± 5.14^{b}	150.78 ± 3.02^{b}	41.07 ± 1.91^{b}	71.33 ± 7.06^{b}
GROUP III	89.52 ± 0.60^{c}	128.00 ± 0.76^{c}	28.26 ± 0.04^{a}	40.23 ± 4.28^{a}
GROUP IV	111.25 ± 9.52^{bc}	146.12 ± 1.21^{bd}	32.68 ± 1.74^{c}	57.16 ± 2.18^{c}
GROUP V	107.06 ± 8.01^{bc}	135.28 ± 1.66^{cd}	34.43 ± 0.05^{c}	57.08 ± 2.04^{c}

Key: Data are expressed as means ± SEM (n = 5). Values with different alphabets within a column are statistically significant at $P < 0.05$. Group I- Normal control, Group II- CCl_4 induced control, Group III- Silymarin, Group IV- 200mg/kg b.wt of *L.inermis* + CCl_4 Group V- 400mg/kg b.wt of *L. inermis* + CCl_4.

and creatinine levels when compared to the group I. Pretreatment with extract, however led to significant reduction ($P < 0.05$) in urea and creatinine levels on day 8 and 14 of the experiment. Results for this experiment are displayed on table 4.

4. Discussion

Inadequate primary healthcare in most rural communities within Africa and especially, Nigeria has resulted in an ever increasing dependence on medicinal plants for the management of diseases. *L. inermis* has been shown to be rich in polyphynols, flavonoids, phenolic acids, lignin, tannins and compounds which posses antiploriferative, antioxidant, antimutagenic and anticarcinogenic potentials [39]. These compounds may help in protecting the liver and kidney by either inhibiting free radical production or scavenging of the radicals. The importance of *L. inermis* in folklore medicine has necessitated investigations into its effect on varying diseased state.

Carbon tetrachloride is utilized in the laboratory for induction of toxic effect on the liver and kidneys. Induction of liver injury using CCl_4 to evaluate hepatoprotective agents has been reported to elevate serum AST, ALT and ALP enzyme activities as well as levels of bilirubin, urea and creatinine while decreasing total protein, albumin and HDL-c levels [16, 40, 41]. The Trichloromethyl radical of biotransformed CCl_4 can combine with cellular proteins and lipids to form peroxyl radicals that compromise biomembrane integrity. The present study reveals elevation of serum ALT and AST activity serum levels of total bilirubin, Total cholesterol, TAG, LDL-c, Urea and Creatinine with concomitant reduction in total proteins, albumin and HDL-c in intraperitoneally induced CCl_4. All values were found to be significant on the 14th day of the experiment with the exception of total bilirubin.

Reduction in serum total protein and albumin levels can serve as indicators of liver damage and therefore decreased synthetic capacity while elevation of bilirubin in the serum is indicative of biliary excretory mechanisms [42]. Animals pretreated with *L .inermis* extract showed significant reversal of the effect of CCl_4 in the parameters investigated at the doses administered. Results obtained for total protein and albumin levels indicate dose dependent increases respectively, while Total bilirubin levels showed dose dependent decreases on day 14 of the experiment. This is in agreement with previous works [26].

The serum levels of aminotransferases in animal treated with extracts showed reduction in serum ALT and AST activities compared with animal that received only CCl_4. Reduction in serum enzyme activity were observed to be dose dependent for animals treated with extracts of *L. inermis*. Findings in these experiment are in agreement with results from previous investigations [26, 43] High values of these serum enzyme markers (ALT and AST activity) are often indicative of conditions such as hepatitis, toxic liver diseases,

Table 3: Effect of extract on Lipid profile.

Treatment	TC 24hrs (mg/d)	TC day 14(mg/dl)	TAG 24hrs(mg/d)	TAG day 14(mg/dl)	HDL 24hrs (mg/dl)	HDL day 14 (mg/dl)	LDL 24hrs (mg/dl)	LDL day 14 (mg/dl)
GROUP I	96.11 ± 8.72^{a}	135.76 ± 10.15^{a}	67.32 ± 3.06^{a}	78.13 ± 5.32^{a}	64.98 ± 11.51^{a}	59.07 ± 5.24^{a}	29.99 ± 7.69^{a}	46.67 ± 5.41^{a}
GROUPII	155.61 ± 13.24^{b}	234.17 ± 3.11^{b}	103.38 ± 8.98^{b}	124.08 ± 8.34^{b}	45.45 ± 3.92^{a}	30.63 ± 1.37^{b}	89.91 ± 16.67^{b}	173.36 ± 7.61^{b}
GROUPIII	107.32 ± 1.13^{a}	158.99 ± 10.80^{ac}	88.17 ± 6.01^{bc}	101.36 ± 5.10^{c}	59.72 ± 6.81^{a}	52.21 ± 4.34^{ac}	43.69 ± 19.13^{ab}	84.51 ± 6.08^{ac}
GROUPIV	130.91 ± 16.89^{ab}	187.05 ± 2.49^{c}	80.24 ± 4.28^{ac}	92.88 ± 3.43^{ac}	47.41 ± 2.22^{a}	36.00 ± 4.43^{b}	58.76 ± 17.64^{ab}	130.23 ± 19.42^{d}
GROUP V	120.16 ± 18.47^{ab}	181.37 ± 13.10^{c}	92.34 ± 4.08^{bc}	91.13 ± 4.87^{ac}	48.71 ± 6.44^{a}	43.38 ± 5.10^{bc}	60.78 ± 21.96^{ab}	120.36 ± 19.15^{cd}

Key: Data are expressed as means ± SEM (n = 5). Values with different alphabets within a column are statistically significant at $P < 0.05$. Group I- Normal control, Group II- CCl_4 induced control, Group III- Silymarin, Group IV- 200mg/kg b.wt of *L.inermis* + CCl_4 Group V- 400mg/kg b.wt of *L. inermis* + CCl_4.

Table 4: Effect of extract on Urea and Creatinine levels.

Treatment	Urea 24hrs (mg/dl)	Urea day 14 (mg/dl)	Creatinine 24hrs (mg/dl)	Creatinine day 14 (mg/dl)
GROUP I	18.80 ± 0.53^{a}	18.80 ± 0.53^{a}	0.83 ± 0.28^{a}	1.67 ± 0.84^{a}
GROUP II	40.91 ± 4.02^{b}	40.95 ± 4.05^{b}	15.33 ± 1.33^{b}	16.00 ± 1.35^{b}
GROUP III	36.76 ± 1.21^{b}	35.74 ± 0.63^{b}	2.17 ± 0.87^{a}	5.07 ± 1.71^{a}
GROUP IV	22.67 ± 1.95^{ac}	22.67 ± 1.95^{ac}	4.33 ± 0.77^{cd}	9.99 ± 1.50^{c}
GROUP V	27.61 ± 1.99^{c}	27.64 ± 1.97^{c}	6.50 ± 1.88^{d}	6.10 ± 1.40^{ac}

Key: Data are expressed as means ± SEM (n = 5). Values with different alphabets within a column are statistically significant at $P < 0.05$. Group I- Normal control, Group II- CCl_4 induced control, Group III- Silymarin, Group IV- 200mg/kg b.wt of *L.inermis* + CCl_4 Group V- 400mg/kg b.wt of *L. inermis* + CCl_4.

acute myocardial infarction and liver necrosis and ALT is one of the plasma cardiac markers that increase sequentially after acute myocardial infarction [16, 44]. A rise in serum ALT and AST activity is also an indicator of extent of damage done to cellular structures like the cytoplasmic and mitochondrial membranes and their relative plasma activities may help to indicate the type of hepatic damage [45].

Results obtained for this experiment investigating the effect of extracts on serum lipid profile reveals a reduction in serum total cholesterol, TAG and LDL-c with concomitant elevation of serum HDL-c. Elevated serum levels of total cholesterol, TAG and LDL-c have been implicated in cardiovascular disorders [46]. Elevation of total cholesterol, triglycerides and LDL-c in this experiment shows that CCl_4 induction may affect normal lipoprotein and cholesterol clearance. On the other hand, pretreatment with plant extracts leading to an elevation of serum HDL-c and reduction in total Cholesterol levels may be indicative of a return to near normal lipid clearance.

Products of purine and protein degradation such as urea and creatinine are elevated in cases involving chemically induced nephrotoxicity [47, 48]. Previous investigations report that chemically induced toxicity resulted in serum elevation of creatinine and Urea levels respectively [48, 49]. These elevations may be as a result of a breakdown in kidney function. Results obtained for the current study showed marked reversals in serum urea and creatinine levels in CCl_4 induced animals pre-treated with ethanol leaves extracts *of L. inermis.*

5. Conclusions

Results for the experiments conducted indicate that pretreatment with *L. inermis* ethanol leaves extract may possess some protective effect by reversing the damage caused by CCl_4 induced liver injury. Serum enzyme levels, lipid parameters, urea and creatinine elevated by CCl_4 induced toxicity were found to be lowered in animals pretreated with plant extract. Conversely, serum albumin, total protein and HDL-c were elevated in pretreated animals. This supports the use of *L. inermis* leaves in folklore medicine in the management of liver and kidney damage. To further understand the potential for protection, experiments involving histopathological studies along with *in vivo* antioxidant effect of *L. inermis* leaves extracts on CCl_4 induced toxicity are been conducted in our laboratory. Also, because *L. inermis* is rich in phytochemicals which contribute vastly to its medicinal properties, we have commenced investigations into isolation, purification, and profiling of active components present in the plant extracts. This we believe will help in increasing our understanding of its molecular mode of action in protecting against liver and kidney damage.

Author Contributions

conceptualization, O.J.; methodology, O.J.; formal analysis, O.J.; investigation, A.P. and A.J.; resources, E.E. and E.A.; data curation, A.P and A.J.; writing—original draft preparation, O.J.; supervision, O.J.; project administration, O.J.

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Competing Interests

The authors declare no competing interests. t

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