Research Article

Investigation on Potential Efficacy of Methanol Extract of *Lawsonia inermis* L. Against Carbon Tetrachloride Induced Hepatotoxicity in Wistar *Albino* Rats

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ABSTRACT

Carbon tetrachloride (CCl_4) a pharmacological tool was used to induce liver damage in Wistar *albino* rats. Silymarin (100 mg/kg) and methanol extract of *Lawsonia inermis* Linn. family Lythraceae (known to possess hepatoprotective compounds) was used to reverse the liver damage caused due to CCl_4 (induced toxicity). Hydroalcholic extract of stem bark and leaf of *Lawsonia inermis* Linn. was evaluated, for its restorative efficacy against CCl_4 induced hepatotoxicity which was assessed in terms of biochemical and histopathological parameters. CCl_4 produce the altitude levels of serum marker enzymes of alanine aminotransferase (ALT), asparate aminotransferase (AST), alanine phosphatase (ALP) and bilirubin in blood serum. Due to the toxicity of CCl_4 cause turbine hepatic cell architecture, necrosis, inflammatory cell infiltration, congestion, and sinusoidal dilatation along with reduction of superoxide dismutase (SOD), catalase, Glutathione-S-transferase (GST) and glutathione peroxidase (GPx) appeared in liver tissue. SOD is the primary step in the defense mechanism involved in the antioxidant system against the oxidative stress. It diminishes by converting the superoxide radical in to peroxide and molecular oxygen. CAT or GPx reactions, also exert a similar effect thereby reducing the level of cellular damage. By oral administration of methanol extract of *Lawsonia inermis* Linn. plant extracts, i.e., stem bark extract (250 mg/kg b.wt.) and leaf extract (250 mg/kg b. wt.) the levels of these parameters was restored to near controlled (untreated) levels. Thus, the present study revealed that the extracts of stem bark and leaf of *Lawsonia inermis* Linn. offered protection against hepatotoxicity induced by CCl_4.

Keywords: *Lawsonia inermis*, Liver marker enzymes, Carbon tetrachloride, Hepatoprotective.

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INTRODUCTION

Hepatic system is a very vital organ system involved in the body's metabolic and biochemical activities. Due to this, the chemical reactions in the liver may produce several reactive species like free radicals. These reactive species form strong bonds with the lipids of the tissue. However, some species do not built protective mechanisms oppose the hazardous reactions associated with the free radicals. Due to over exposure to toxic chemicals, the formation of free radicals will be so high that they overpower the natural defense system induce hepatic damage and cause jaundice, cirrhosis, fatty liver and abnormal architecture of the hepatic cells, which remain one of the serious health problems. Carbon tetrachloride (CCl_4) is one such hazardous chemical which induces hepatotoxicity through membrane lipid peroxidation by its free radical derivatives, trichloromethyl radical (CCl_3) and trichloromethylperoxy radical (CCl_3O_2). Excessive production of the reactive species manifests in tissue thiol depletion, lipid peroxidation, plasma membrane damage etc., culminating in severe hepatic injury [1-2]. This can be controlled by the traditional systems of herbal medicine continue to play a vital role in the health care system of the population. [3-4]*Lawsonia inermis Linn.* is regularly used by the rural and tribal people in curing various health disorders. [5]

Many of the antitumour drugs generate free radicals; they are known to produce myelosupression, nephrotoxicity and hepatotoxicity. [6] *Lawsonia interims* Linn. was used for treatment of cancer. [7] Besides its use in cosmetics for staining hands and producing hair dye, the leaves are used as a prophylactic against skin diseases. [8] *Lawsonia inermis* Linn. is reported to have anti-inflammatory, antipyretic and analgesics properties. [9-10] The bark extract of this plant is an appetiser, anti-inflammatory, used in liver disorders, fractures and also reported to be useful in jaundice, splenomegaly,[10-13] calculus affliction, and skin diseases. [14] The leaf extract of *Lawsonia inermis* has been shown to possess antimicrobial[15] and antitubercular activity. Ethanol extract of the whole plant was found to have antifungal activity. [16] An ointment prepared from the leaves was used to cure ulcers and wounds. [17] The decoction of bark and

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leaves has been found to inhibit the peptic enzymes. [18] The methanol extract of bark was found to alleviate the levels of liver marker enzymes and restore normal bile flow. [19] CCl_4 induced hepatotoxicity depends on the reductive dehalogenation of CCl_4 catalyzed by Cyt P450 in the liver cell endoplasmic reticulum [20] leading to the generate an unstable complex of CCl_3 radical. This trichloromethyl radical reacts rapidly with O_2 to yield trichloromethyl peroxy radical, which is reported as a highly reactive species. Almost all parts of the plants were being used as a medicine to prevent and cure many diseases and for other purposes. [21,22,23] Nowadays attention is being focused on herbal medicines because of their effectiveness, easily available, low cost and for being comparatively devoid of side effects. The present study was designed with an aim of assessing the hepatoprotective activity by the stem bark and leaf extract of *Lawsonia inermis Linn.* against CCl_4 induced liver damage. Scavengers of free radicals can reduce side effects of these drugs. Plant kingdom possess several non-toxic compounds that can scavenge free radicals and boost the antioxidant defense mechanism in the body and have a protective and curative role against tissue damage induced by chemicals and drugs. [24-29]

MATERIALS AND METHODS

Plant material

The leaves of *Lawsonia inermis* were collected in and around Vellore District, Tamilnadu, and authenticated at the Department of Botany, C. Abdul Hakeem College, Melvisharam, Vellore Dt, Tamil Nadu. Voucher specimen was deposited at the Institutes' Herbarium.The collected fresh plants were washed thoroughly 2-3 times in running tap water and once again with sterile water and then stained it well and shade-dried at room temperature without any contamination. The dried stem bark and leaves were collected in separately and then powdered using a grinder.

Plant extract preparation

The shade dried plant materials were powdered (stem bark and leaf) separately in an electrical blender. The powdered material of the stem bark and leaf were extracted for 3 days with Soxhlet extraction by using methanol (500 ml for 100 gms) as solvent. The extracts was filtered and dried separately under reduced pressure on rotary evaporator to obtain (10%) of each extract. The obtained powder was then subjected to phytochemical analysis to determine the chemical constituents present in the extract and the remaining was stored at 5°C for further use.

Animals

Wistar *albino* rats, male (175-200g) were obtained from the Institute's Animal House, C. Abdul Hakeem College, Melvisharam, Tamil Nadu, India. They were housed under standard conditions (temperature 25-27°C, relative humidity 60-70% and 12 hr dark-light cycles), fed with commercial rat feed (Lipton India Ltd., Mumbai, India) and boiled water ad libitum. In this study, experimental protocol and procedures employed were approved by the Animal Ethics Committee of C. Abdul Hakeem College, Melvisharam, Tamil Nadu, India (No.1011/c/06/CPCSEA). The rats were kept and monitored in animal house for ten days before starting the experiment.

Experimental design

Group I: Normal rats – Treated with common diet and water.

Group II: Rats injected 30% CCl_4 with olive oil (1 ml/kg body wt., by intra peritoneal administration) every 72 hrs for 3 successive doses.

Group III: The CCl_4 injected rats were treated with silymarin (100 mg/kg b.wt.) orally by intra gastric tube for 30 days.

Group IV: The CCl_4 injected rats were treated with methanol stem bark extract (250 mg/kg b.wt.) of *Lawsonia inermis* Linn. orally, by intra gastric tube for 30 days.

Group V: The CCl_4 injected rats were treated with methanol leaf extract (250 mg/kg b. wt.) of *Lawsonia inermis* Linn. orally, by intra gastric tube for 30 days.

The animals were sacrificed under the light ether anesthesia. Blood was drawn by cardiac puncture and serum samples were obtained by centrifuging all blood at 3000 rpm and 4°C. Hepatic tissue was homogenized in suitable buffer and centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was collected and stored at -80°C for assay of the marker enzymes and antioxidant studies.

Assessment of hepatoprotective activity

Hepatic enzymes, AST and ALT were used as the biochemical markers of the hepatic cell damage and were assayed by the method of. [30] ALP activity was measured using the method of [31], and serum bilirubin was estimated by the method of [32], to assess the acute hepatic damage caused by CCl_4.

Assay of superoxide dismutase activity (SOD)

The activity of superoxide dismutase (SOD) was measured by the modified method of. [33] The liver homogenate containing about 5µg of protein- was mixed with sodium pyrophosphate buffer, PMT and NBT. The reaction was initiated by the addition of NADH. Then, the reaction mixture was incubated at 30°C for 90s. Next, the reaction was stopped by the addition of 1 ml of glacial acetic acid. The absorbance of the resultant chromogen was measured at 560 nm. One unit of SOD activity is defined as the enzyme concentration required to inhibit chromogen production by 50% in 1min under the assay condition.

Assay of Catalase (CAT)

The CAT activity was measured in liver homogenates by the method of. [34] For the assay, the liver homogenates containing 5 µg total proteins were mixed separately with

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700 µl, 5mM hydrogen peroxide and incubated at 37°C. The disappearance of peroxide was observed at 240 nm for 15 min. One unit of catalase activity is that which reduces 1µmol of hydrogen peroxide per minute.

Assay of Glutathione-S-transferase (GST)

Glutathione-S-transferase activity was estimated by the method of. [35] The reaction mixture consisted of 2.75 ml sodium phosphate buffer (0.1M, pH 7.4), 0.1 ml reduced glutathione (1mM), 0.1ml PMS (10% w:v) in a volume of 3.0 ml. The changes in the absorbance were recorded at 340 nm and the activity of the enzymes was calculated as nmol CDNB conjugate formed/ min/ mg protein using a molar coefficient of 9.6_103:M:cm.

Statistical analysis

The obtained data were expressed as mean ± standard deviation (SD) and analyzed using one-way analysis of variance (ANOVA), followed by Scheffe post hoc test. The data were analyzed with SPSS version 16 software (SPSS Inc., Chicago, USA). Statistical significance of difference was accepted at the p-values of less than 0.05.

RESULTS

Effect of *Lawsonia inermis* on CCl_4-induced hepatotoxicity

The results of CCl_4-induced hepatotoxicity were summarised in Table 1. CCl_4 -treated group revealed a significant elevation of serum marker enzyme activities of ALT (98.90%), AST (78.38%), ALP (83.24%) and bilirubin (83.58%) as compared to normal control ($p < 0.05$), which indicates that CCl_4 induced damage to the hepatic cells. However, treatment with *Lawsonia inermis* stem bark at a dose of 250 mg/kg had significantly decreased the elevated percentages of ALT, AST, and ALP and bilirubin by 43.63%, 40.76%, 41.27%, and 39.83% respectively as compared with CCl_4 treated group. Similarly, methanol leaf extract of *Lawsonia inermis* at a dose of 250 mg/kg had significantly decreased the elevated percentages of ALT, AST, and ALP and bilirubin by 41.41%, 38.34%, 40.00%, and 35.77% respectively when compared with CCl_4 treated group. Positive control drug, silymarin, at dose of 100 mg/kg also reduced the levels of serum ALT, AST, and ALP and bilirubin (47.53%, 42.33%, 43.41% and 41.46%) respectively (Table.1).

Effect of *Lawsonia inermis* on hepatic antioxidant enzyme activities

The natural antioxidant enzymes are the defense system of the body. The level of the antioxidants (SOD, catalase, GST and GPx) exhibits the real scenario of the body. The activities of liver SOD, catalase, GPx and GST in CCl_4 treated group were significantly decreased by 51.45%, 47.76%, 53.06%, and 41.34% respectively, when compared with the normal control group. Methanol extract of *Lawsonia inermis* stem bark showed a significant enhancement in the activities of SOD, CAT, GPx and GST to 94.87%, 77.62%, 106.94%, and 57.46% respectively compared to the CCl_4 treated group. Similarly administration of the methanol leaf extract of *Lawsonia inermis* showed a hike in the levels of the SOD, catalase, GPx and GST (71.79%, 73.99%, 100.21% and 47.61%) reduced by the CCl_4. Administration of silymarin also significantly increased ($p < 0.05$) the activities of SOD, CAT, GPx and GST (100.85%, 86.27%, 110.73%, and 62.53%) respectively reduced by the CCl_4 compared to the CCl_4-treated group (Table 1).

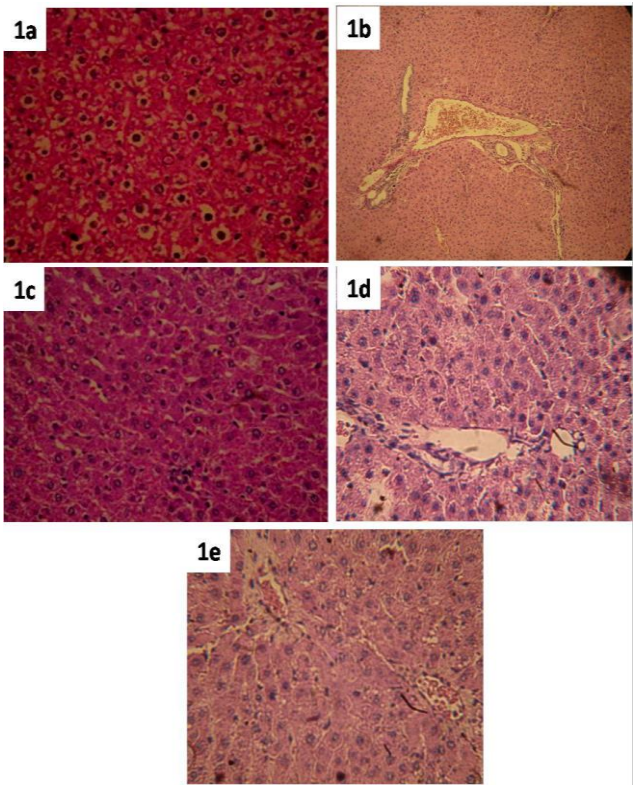

Figure 1: Histopathological changes in liver tissues stained with H&E.

1a - Normal, 1b - CCl_4 induced cell, 1c - CCl_4 + Sylimarin treated cell, 1d - CCl_4 + 250 mg/kg body wt., *Li* bark extract treated, 1e - CCl_4 + 250 mg/kg body wt., *Li* leaf extract treated

Histological examinations

Histological studies also provided a supporting evidence for the biochemical analysis. In normal control animals, liver sections showed normal hepatic cell architecture with well preserved cytoplasm, prominent nucleus and nucleolus and central vein (Fig. 1a). The leaves extract treated rats revealed moderate inflammatory cell infiltration of hepatocytes, moderate necrosis, mild ballooning, degeneration, and mild fibrosis (Fig. 1e), compared with the lesions observed in the CCl_4 control group (Fig. 1b). The lesions of silymarin treated rats showed traces of mild diffused necrosis of hepatocytes, mild inflammatory cell infiltration, trace of ballooning degeneration, and mild fibrosis (Fig. 1c). On the other hand, a trace of mild degree of hepatocellular necrosis, inflammatory cell and hepatocyte fibrosis, and mild degree of balooning, degeneration were observed in the liver of extracts of *Lawsonia inermis* stem bark (250 mg/kg b.wt.) treated rats (Fig. 1d). Fig.1e. shows a nearly normal cell, which was treated with leaf extract of *Li*.

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Table 1: Effect of bark and leaf extract on CCl_4 induced hepatotoxicity: Levels of ALT, AST, ALP, BR, SOD, CAT, GPx, and GST

Groups	Parameters in the serum				Parameters in liver tissue			
	ALT (IU/l/min/mg protein)	**AST** (IU/l/min/mg protein)	**ALP** (IU/l/min/mg protein)	**Bilirubin** (mg/dl)	**SOD** (U_1/mg protein)	**CAT** (U_2/mg protein)	**GPx** (U_3/mg protein)	**GST** (U_4/mg protein)
Group-I (Normal)	52.15±1.51	69.22±0.98	77.35±1.32	0.67±0.26	2.41±1.14	80.33±0.94	10.12±1.06	5.37±0.42
Group-II (CCl_4 control)	103.73±1.15[a]	123.48±1.96[a]	141.74±1.11[a]	1.23±0.75[a]	1.17±1.12 [a]	41.96±1.41[a]	4.75±1.15[a]	3.15±1.02[a]
% of change (Normal vs CCl_4)	**+98.90**	**+78.38**	**+83.24**	**+83.58**	**-51.45**	**-47.76**	**-53.06**	**-41.34**
Group-III (CCl_4 + Silymarin 100 mg/ kg body wt.,)	54.42±1.27[b]	71.21±1.04[b]	80.21±0.75[b]	0.72±0.48[b]	2.35±1.07[b]	78.16±1.11[b]	10.01±1.24[b]	5.12±0.54[b]
% of change (CCl_4 + vs silymarin)	**-47.53**	**-42.33**	**-43.41**	**-41.46**	**+100.85**	**+86.27**	**+110.73**	**+62.53**
Group –IV CCl_4 +*Li* (bark 250 mg/ kg body wt.,)	58.47±1.03[b]	73.14±1.05[b]	83.24±1.44[b]	0.74±0.68 [b]	2.28±1.16[b]	74.53±1.25[b]	9.83±1.55[b]	4.96±1.21[b]
% of Changes CCl_4 vs *Li* (bark)	**-43.63**	**-40.76**	**-41.27**	**-39.83**	**+94.87**	**+77.62**	**+106.94**	**+57.46**
Group –V CCl_4 +*Li* (leaf 250 mg / kg body wt.,)	60.77±1.13[b]	76.13±1.21[b]	85.03 ±1.30[b]	0.79±0.87[b]	2.01±1.53[b]	73.01±1.23[b]	9.51±0.41[b]	4.65±0.16[b]
% of Changes CCl_4 vs *Li* (leaf)	**-41.41**	**-38.34**	**-40.00**	**-35.77**	**+71.79**	**+73.99**	**+100.21**	**+47.61**

1.Values are mean of six individual observations in each group ±S.D.

2. 'P' denotes statistical significance. P<0.05. '+' and '-'indicates % of changes over the CCl_4 intoxicated groups. a- compared with normal, b-compared with CCl_4.

3. SOD – U_1- One unit of activity was taken as the enzymes reaction which gives 50% inhibition of NBT reduction in one minute. CAT – U_2- µmoles of hydrogen peroxide consumed per minute. GPx – U_3- µg of glutathione consumed per minute. GST – U_4- µmoles of CDNB – GSH conjugate formed per minute.

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DISCUSSION

Carbon tetrachloride is a xenobiotic that produces hepatotoxicity in experimental animals and human beings and its biotransformed metabolites (by Cytochrome P-450 (CYP)) such as trichloromethyl radical (CCl_3) and trichloromethyl peroxyl radical (CCl_3O_2) were reported to initiate peroxidation [20] and is involved in the pathogenesis of liver. [36] Both free radicals were capable of binding to proteins and lipids, leading to membrane lipid peroxidation and finally cell necrosis. [37-20] Many studies have shown that a crucial mechanism of the hepatoprotective effects may be related to the ability of antioxidants to scavenge reactive oxygen species. [38]

In the present study, we found that treatment with the *Lawsonia inermis* significantly inhibited CCl_4-induced liver damage as evidenced by decreased serum activities of AST, ALT, and ALP, and bilirubin. To prevent the oxidative damage, tissues have constructed an antioxidant defense system that includes enzymatic antioxidants and non-enzymatic antioxidants. [39-40] Therefore, the activities of enzymatic antioxidants inhibit the generation of free radicals. Hence the hepatic cells are protected from CCl_4 induced liver damage. [41] SOD is a manganese containing enzyme in mitochondria which converts the dismutation of superoxide anions into hydrogen peroxide (H_2O_2). [42] Catalase is a hemoprotein in all aerobic cells that decomposes H_2O_2 to oxygen and water. GSH and GPx metabolize H_2O_2 and hydroperoxides to non-toxic products and terminate the chain reaction of lipid peroxidation by removing lipid hydroperoxides from the cell membrane. [43] GSH-Rd is a cytosolic hepatic enzyme involved in the detoxification of a range of xenobiotic compounds by their conjugation with GSH. [44,45,46] These antioxidant enzymes are effortlessly inactivated by lipid peroxides or free radicals, which results in decreased activities of these enzymes in CCl_4 toxicity. The results of the present study indicate that SOD, catalase GPx and GST activities were significantly decreased in CCl_4 treated liver, compared with normal control rats, implying increased oxidative damage to the liver. On the contrary, SOD, catalase, GPx and GST levels were significantly elevated by administration of *Lawsonia inermis* and sylimarine in CCl_4-induced rats, suggesting that it has the ability to restore/ maintain the activity of hepatic enzymes in CCl_4-damaged liver.

Histopathological observations such as hepatocyte necrosis, inflammatory cell infiltration, ballooning degeneration, and hepatocyte fibrosis were recorded. Through semi-quantitative assessment, all scores of histopathologic examinations in the CCl_4-treated group were significantly higher than that of the normal control ($p< 0.05$), indicating that CCl_4 has induced severe damage to the hepatic cells. The extract of stem bark and leaf extracts of *Lawsonia inermis* significantly decreased ($p< 0.05$) the scores of hepatocyte necrosis and inflammatory cell infiltration as compared to CCl_4-treated group. Similar cell architecture was observed in animals treated with silymarin when compared to normal. Microscopic examinations confirmed that severe liver damages induced by CCl_4 were remarkably reduced by the administration of methanol extract of stem bark and leaf, which was in good correlation with the results of the serum aminotransferase and hepatic antioxidant enzyme activities. According to the results obtained, it may be concluded that all the fractions of the methanol extract of the *Lawsonia inermis* (stem bark and leaf) proved to have strong antioxidant activity, reducing power. ability, and free radical scavenging activity. Among these two extracts, extract of bark revealed significant curative effect than extract of leaves.

CONCLUSION

The methanol extract of *Lawsonia inermis* (stem bark and leaf) exhibits hepatoprotective and curative effects on CCl_4 induced liver damages. Treatment with 250 mg/kg of *Lawsonia inermis* (stem bark and leaf) exhibited optimal protection on the CCl_4 induced hepatotoxicity and nearly similar hepatoprotective and curative effects like silymarin. *Lawsonia inermis* contains several chemical compounds like alkaloids, saponins, titerpenoid etc. These might be responsible for the hepatoprotective and curative activity in the CCl_4 induced rats.

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