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HEPATOPROTECTIVE POTENTIAL OF *LAWSONIA INERMIS* L. (SEEDS)

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ABSTRACT

This study aimed to assess the hepatoprotective activity of ethanol (90%) extract and its ethyl acetate fraction *Lawsonia inermis* L. seeds on carbon tetrachloride (CCl_4) induced hepatotoxicity in rats and to ascertain the claim of its use in liver disorders. Pre-treatment of rats with doses of 200 and 400 mg/kg, b.wt; p.o. of the ethanol extract and its ethyl acetate fraction significantly ($P < 0.05$) lowered serum transaminases (AST and ALT), alkaline phosphatase (ALP), total bilirubin (TB) and increased the levels of the total proteins (TP) and albumin (ALB) respectively, in a dose dependent manner against the significant ($P < 0.01$) alteration of these damaged marker enzymes when challenged with CCl_4 (0.5 ml/kg, i.p.). Parallel to these changes, the seeds extract and its fraction prevented CCl_4 induced oxidative stress by significantly restoring the levels of reduced glutathione and lowering the levels of hepatic malondialdehyde by inhibiting the production of free radicals. These biochemical parameters were supplemented by histopathological examination of liver sections. Taken collectively, these findings suggest that ethyl acetate fraction revel more significant ($P < 0.05$) hepatoprotective potential against CCl_4 induced hepatotoxicity in rats and confirms the folklore use of this plant.

Key words: Carbon tetrachloride; Hepatoprotective; *Lawsonia inermis*; Oxidative stress; Transaminases.

INTRODUCTION

Lawsonia inermis L. (Lythraceae) a shrub commonly known as '*Mhendi*' and '*Henna*', indigenous to North Africa, South-West Asia and India (Sastri, 1962). Besides its use in cosmetics and as a hair dye, the henna is also used as a prophylactic against skin diseases and inflammations, hepatoprotective, tuberculostatic, fungicidal (Ahmed *et al.*, 2000), immunostimulant (Mikhaeil, 2004), memory enhancer (Iyer *et al.*, 1998), hypoglycaemic (Syamsudin and Winarno, 2008), cytotoxic (Endrini *et al.*, 2002) and bactericidal (Ali *et al.*, 2001).

Liver disorders are one of the major health problems in human due to various chemicals (Carbon tetrachloride, D-galactosamine, thioacetamide) including therapeutic agents (acetaminophen) and other environmental toxins that can produce hepatotoxicity which may even leads to death (Victor *et al.*, 2006). CCl_4 hepatotoxicity depends on the reductive dehalogenation of CCl_4 catalyzed by Cyt P450 in the liver cell endoplasmic reticulum leading to the generation of an unstable complex of $CCl_3^{\cdot}$ radicals. This trichloromethyl radical reacts rapidly with O_2 to yield trichloromethyl peroxy radicals which is reported as a highly reactive species. These free radical attacks microsomal lipids leading to its peroxidation and also covalently binds to microsomal lipids and proteins ultimately initiating a site of secondary biochemical processes which is the ultimate cause for the unfolding of the panorama of pathological consequences of CCl_4 metabolism (Kapur *et al.*, 1994). Hepatic injury is an increase of more than three times of normal serum AST and ALT; an ALP and total bilirubin more than two times of normal (Victor *et al.*, 2006).

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Malondialdehyde (MDA) is a major reactive aldehyde resulting from the peroxidation of biological membrane of polyunsaturated fatty acid and is used as an indicator of tissue damage involving a series of chain reactions (Ohkawa *et al.,* 1979). Deficiency of GSH within living organisms can lead to tissue injury and disorders. It is a non-protein thiol and intra-cellular reductant which plays major role in catalysis, metabolism and transport. It protects cells against free radicals, peroxides and other toxic compounds (Gupta *et al.,* 2004).

Natural products are the best source of prophylactic treatment of liver disorders. Thus, identification of a potential therapeutic agent for the protection of liver from the hepatotoxins will provide a useful way for the prevention of these liver related illnesses. According to the literature survey there is no scientific report available in support of the hepatoprotective activity of the seeds of *Lawsonia inermis* L. Therefore, to justify the traditional claims; the seeds of *Lawsonia inermis* L. has been selected for hepatoprotective effect against CCl_4 intoxicated rats.

MATERIALS AND METHODS

Plant material

Lawsonia inermis L. seeds were collected in the month of June from regional areas of Salem, Tamilnadu and authenticated by Dr. K.M. Chetty, Asst. Prof., Deptt. of Botany, Sri Venkateswara Uni., Tirupati and Dr. H.B. Singh, Scientist F & Head, Raw Material Herbarium & Museum, National Institute of Science Communication and Information Resources (NISCAIR) New Delhi, India (Voucher specimen - NISCAIR/RHMD/Consult/-2008-09/1175/207). The seeds were shade dried, coarsely powdered and stored in an air tight container till use.

Extraction and fractionation

The seeds were defatted with *n*-hexane. The dried solvent freed marc was extracted by cold maceration with ethanol (90%) till exhausted completely. The ethanol extract so obtained was freed off solvent under vacuum and further extracted with ethyl acetate by sonication.

Phytochemical screening

Screening of phytoconstituents present in the ethanol extract and its ethyl acetate fraction were carried out by using respective testing reagents (Harbone, 1998).

Screening for hepatoprotective potential

Drugs and Chemicals/Instruments

Reduced glutathione (GSH), malondialdehyde (MDA), sodium dodecyl sulphate (SDS), thiobarbituric acid (TBA), trichloroacetic acid (TCA), disodium ethylene diamine tetra acetic acid (EDTA), 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB), tris-HCl, sodium citrate, acetic acid, butanol, pyridine, carbon tetrachloride (CCl_4), olive oil, silymarin were procured from Sigma (MO, USA), CDH (New Delhi, India) and Microlabs (Mumbai, India), Cooling Centrifuge (REMI, Vasai), Rotary evaporator (Equitron Roteva, Mumbai), Ultrasonic Cleaner (Steryl Med Equip systems) and UV/Visible Spectrophotometer (UV 1700, Pharmaspec, SHIMADZU, Japan) were used.

Preparation of test material

The ethanol extract and its ethyl acetate fraction were suspended separately in distilled water to a concentration of 50 mg/ml of respective suspension/solution. CCl_4 was suspended in olive oil in 1:1 (Sengottuvelu *et al.,* 2007).

Experimental animals

Albino Wistar rats (either sex) weighing 180-220 g were procured from CPCSEA (Reg. No. 816/04/C) approved animal house of I.S.F. College of Pharmacy, Moga and used throughout the experiment. The animals were housed in an air conditioned room (24±4ºC) with 12-12 h light & dark cycles; had access to a standard chow diet (Ashirwad Industries, Ropar, India) and water *ad libitum*.

Experimental groups

The animals were divided into seven groups consisting of six animals per group. Group I served as vehicle control and received distilled water. Group II as disease control and was given CCl_4 (0.5 ml/kg, b.wt; i.p.) with olive oil in the ratio 1:1. Group III was given daily treatment of standard drug (silymarin at 50 mg/kg, b.wt; p.o.) for 7 days and then toxicated with CCl_4 on day 7^{th}. Group IV consisted of daily treatment of the ethanol extract at 200 mg/kg, b.wt; p.o. for 7 days and then toxicated with CCl_4 on day 7^{th}. Group V was pretreated with the ethanol extract at 400 mg/kg, b.wt; p.o. for 7 days and then toxicated with CCl_4 on day 7^{th}. Group VI was given daily treatment of the ethyl acetate fraction at 200 mg/kg, b.wt; p.o. for 7 days and then toxicated with CCl_4 on day 7^{th}. Group VII rats were pretreated with the ethyl acetate fraction at 400 mg/kg, b.wt; p.o. for 7 days and then toxicated with CCl_4 on day 7^{th}. All the animals were sacrificed 24 h after CCl_4 administration by cervical dislocation. The liver was quickly removed and perfused immediately with ice-cold saline (0.9% NaCl) and homogenised for estimation of tissue parameters. Just before sacrifice, blood samples were collected by retro orbital sinus puncture for the estimation of various serum parameters (Sengottuvelu *et al.,* 2007).

Estimation of serum parameters

The AST (IFCC, 1986), ALT (IFCC, 1986), ALP (Kind and King, 1954), total bilirubin (Jendrassik

and Grof, 1938), total proteins (Doumas, 1975) and albumin (Doumas and Watson, 1971) levels in the serum were estimated using commercially available kits of *Carol* Company, Goa.

Estimation of tissue parameters (Antioxidative effects)

Lipid peroxidation: It was determined by measuring the amounts of malondialdehyde (MDA) produced primarily, according to the method of Ohkawa's 1979. The levels of lipid peroxides were expressed as nM of thiobarbituric acid reactive substances (TBARS)/mg protein (Gupta *et al.,* 2004).

Estimation of GSH: It was determined by measuring the amounts of reduced glutathione (GSH) level, according to the method of Ellman's 1959. The levels of reduced glutathione were expressed as µM of reduced glutathione/mg protein (Gupta *et al.,* 2004).

Histological analysis of liver

Liver tissue of the treated and non treated rats were isolated and fixed in 10% phosphate buffered formalin for at least 24 h. Then the paraffin sections were prepared and cut into 5 µm thick sections in a rotary microtome and mounted on the slide. The sections were then stained with Haematoxylin–Eosin dye and were studied for histopathological changes, i.e. necrosis, fatty changes, ballooning degeneration, lymphocytes and Kupffer cells infiltration. The severity of liver alteration was semi-quantitatively graduated on a scale of 0 to IV (0 = absent; I = minimal; II = mild; III = modest; IV = severe) (Krajian, 1963).

Hepatoprotective activity

The hepatoprotective activity, expressed as hepatoprotective percentage (H), was calculated as follows:

$H = 1 – (T – V/ C – V) \times 100$

Where T is mean value of drug and CCl_4, C mean value of CCl_4 alone and V is the mean value of normal control animals (Chandan *et al.,* 2007).

Safety evaluation study

The safety study was carried out using OECD guide lines/423. Three male rats of same age group and weight were taken in a single dose up to the highest dose of 2000 mg/kg orally. The animals were observed for 1 h continuously and hourly for 4 h and finally after every 24 h up to 15 days for any mortality or gross behavioural changes (Chandan *et al.,* 2007).

Statistical analysis

Results were reported as means ± SD. One Way ANOVA was used to evaluate differences between groups. The differences among the means were analysed by Tukey-Kramer test for multiple comparisons using computerized program at 95% (a, b = $P < 0.01$ and c, d = $P < 0.05$) confidence level.

RESULTS

The yield of the ethanol extract of *Lawsonia inermis* (seeds) was 19.2% w/w and its ethyl acetate fraction was found to be 81.25% w/w by sonication, which is around three times more than the yield (28.6% w/w) obtained by conventional solvent fractionation (Table 1).

Phytochemical investigations revealed the presence of carbohydrates, proteins, alkaloids, phenolic compounds and flavonoids in the ethanol extract. Ethyl acetate fraction extracted from ethanol extract showed the presence of phenolic compounds and flavonoids.

The oral administration of the ethanol extract and ethyl acetate fraction the seeds caused neither any behavioural changes nor mortality up to 2000 mg/kg.

Table 2 shows the levels of serum enzymes (AST, ALT and ALP), total bilirubin, total proteins and albumin in normal group of rats, *viz*., AST (60.61±4.654 U/L), ALT (50.11±3.546 U/L), ALP (163.30±5.399 U/L), TB (0.28±0.096 mg/dl), TP (6.30±0.518 g/dl) and ALB (3.43±0.422 g/dl).

The CCl_4 (0.5 ml/kg; i.p.) challenged group of rats showed increase in the serum levels of enzymes AST (175.80±5.274 U/L), ALT (167.20±3.645 U/L), ALP (275.40±4.867 U/L), TB (0.70±0.169 mg/dl) and reduction in the serum levels of TP (3.27±0.788 g/dl) and ALB (2.03±0.159 g/dl) indicating the development of hepatotoxicity caused by CCl_4 (Table 2).

Pretreatment of rats with the ethanol extract at 200 and 400 mg/kg showed reduction in the rise of AST (29.2%↓ and 58.0%↓), ALT (35.5%↓ and 66.1%↓), ALP (10.8%↓ and 41.4%↓), TB (37.2%↓ and 48.6%↓) and showed the increase in the level of TP (27.3%↑ and 69.4%↑) and ALB (18.2%↑ and 47.8%↑) respectively in comparison to CCl_4 control group. The groups of rats pretreated with the ethyl acetate fraction at 200 and 400 mg/kg showed reduction in the rise of AST (48.7%↓ and 74.8%↓), ALT (54.5%↓ and 74.3%↓), ALP (31.7%↓ and 51.7%↓), TB (43.4%↓ and 57.7%↓) and showed the increase in the level of TP (48.4%↑ and 82.9%↑) and ALB (36.6%↑ and 62.0%↑) respectively in comparison to CCl_4 control group. Pretreatment of group of rats with silymarin at 50 mg/kg showed reduction in the rise of AST (92.7%↓), ALT (95.0%↓), ALP (74.5%↓) and TB (67.8%↓) and increase in the level of TP (97.0%↑) and ALB (74.1%↑) under the same investigational conditions in comparison to CCl_4 control group (Table 2).

Table 2 shows the increase in MDA level in CCl_4 control group (50.47±4.088 nM/mg protein) compared to normal group (20.00±2.988 nM/mg protein). Pretreatment of groups of rats with the ethanol extract and ethyl acetate fraction at 200 and 400 mg/kg showed reduction in the rise of MDA level by 6.60%↓, 24.1%↓ and 15.8%↓, 37.3%↓ respectively compared to CCl_4 control group. The group pretreated with silymarin at 50 mg/kg showed 62.0%↓ reduction of MDA level with reference to CCl_4 control group.

Figure 1 shows the histopathology of liver of normal arrangement of hepatocytes with clear nucleus, central vein and portal triad. Sinusoids at the periphery or the lobule are fused into a reticulum. The hepatocytes are arranged in a series of branching and anatomising perforated laminae to form a labyrinth, between which were sinusoidal spaces. The cytoplasm of the hepatocytes was clearly eosinophilic with prominent nuclei in normal group. Microscopical examination of CCl_4 treated liver

Table 2 shows the reduction in glutathione (GSH) content in the liver homogenate of CCl_4 control group (48.50±4.037 µM/g of liver) as compared to the normal group (106.20±7.360 µM/g of liver). Pretreatment of groups of rats with the ethanol extract and ethyl acetate fraction at 200 and 400 mg/kg showed increase in GSH level by 8.4%↑, 24.9%↑ and 12.8%↑, 48.3%↑ respectively as compared to CCl_4 control group. Pretreatment of rats with silymarin at 50 mg/kg showed increase in the level of GSH by 67.1%↑ under the same investigational conditions in comparison to CCl_4 control group.
section showed various degrees of pathological changes starting from cloudy swelling, hydropic changes and necrosis of hepatic cells as well as centrilobular fatty changes with clear space representing fatty material or lipid. Liver sections of the group of rats pretreated with the ethanol extract and ethyl acetate fraction of the seeds showed the absence of necrosis and vacuoles. The liver sections of the group pretreated with silymarin did not showed parenchymal injury.

Figure 1. Histopathological studies provided supportive evidence for the biochemical analysis
KC- Kupffer cells, HC- Hepatic cells, PV- Portal veins

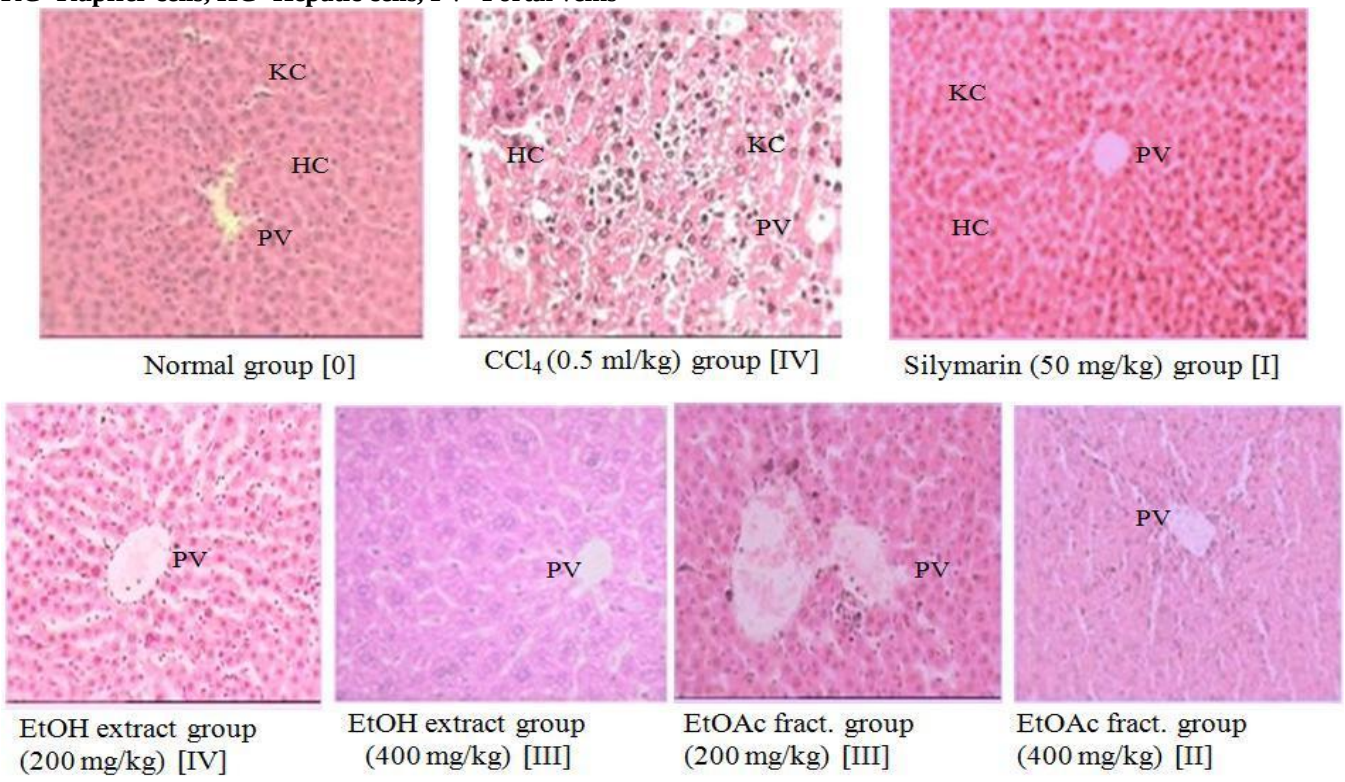

Table 1. Comparison between the yields obtained from the conventional solvent fraction and fractionation by sonication

Ethanol (90%) extract (19.2 g)	Conventional Solvent fractionation	Fractionation by sonication
Ethyl acetate fraction	5.50 g (28.60% of EtOH extract)	15.60 g (81.25 % of EtOH extract)

Table 2. Ethanol extract and its ethyl acetate fraction of *Lawsonia inermis* L. (Seeds) at dose of 200 mg/kg and 400 mg/kg b.wt as hepatoprotective against CCl_4 induced hepatotoxicity in rats

	Serum parameters						Tissue parameters	
Groups	AST (U/L)	ALT (U/L)	ALP(U/L)	TB(mg/dl)	TP(g/dl)	ALB(g/dl)	TBARS (nM/mg protein)	GSH (µM/mg protein)
Normal (Vehicle control)	60.61±4.654	50.11±3.546	163.30±5.399	0.28±0.096	6.29±0.518	3.43±0.422	20.00± 2.988	106.20± 7.360
CCl_4 control (0.5 ml/kg b. wt., i.p.)	175.80±5.274[a]↑	167.20±3.645[a]↑	275.40±4.867[a]↑	0.70±0.169[a]↑	3.27±0.788[a]↓	2.03±0.159[a]↓	50.47± 4.088[a]↑	48.50± 4.037[a]↓
Silymarin (50 mg/kg b. wt., p.o.)	69.02±3.546 (92.7%)[b]↓	56.01±2.205 (95.0%)[b]↓	191.90±4.962 (74.5%)[b]↓	0.41±0.118 (67.8%)[b]↓	6.20±0.610 (97.0%)[b]↑	3.07±0.416 (74.1%)[b]↑	31.60± 1.600 (62.0%)[b]↓	87.17± 4.446 (67.1%)[b]↑
EtOH extract (200 mg/kg b wt., p.o.)	142.20±3.050 (29.2%)[c]↓	125.66±3.071 (35.5%)[c]↓	263.30±3.967 (10.8%)[c]↓	0.54±0.084 (37.2%)	4.10±0.388 (27.3%)[c]↑	2.28±0.103 (18.2%)	48.48± 3.342 (6.6%)	52.33± 2.160 (8.4%)
EtOH extract (400 mg/kg b wt., p.o.)	109.00±6.281 (58.0%)[c]↓	89.81±3.388 (66.1%)[c]↓	229.00±4.489 (41.4%)[c]↓	0.50±0.018 (48.6%)[c]↓	5.37±0.080 (69.4%)[c]↑	2.70±0.081 (47.8%)[c]↑	43.15±1.033 (24.1%)[c]↓	62.83±3.430 (24.9%)[c]↑
EtOAc fract. (200 mg/kg b wt., p.o.)	119.80±5.089 (48.7%)[c]↓	103.45±3.791 (54.5%)[c]↓	239.90±4.325 (31.7%)[c]↓	0.52±0.023 (43.4%)[c]↓	4.83±0.063 (48.4%)[c]↑	2.54±0.043 (36.6%)[c]↑	45.68±1.990 (15.8%)	55.83±2.483 (12.8%)
EtOAc fract (400 mg/kg b wt., p.o.)	89.65±5.023 (74.8%)[c, d]↓	80.29±3.979 (74.3%)[c, d]↓	217.50±3.960 (51.7%)[c, d]↓	0.46±0.021 (57.7%)[c]↓	5.77±0.063 (82.9%)[c]↑	2.90±0.060 (62.0%)[c]↑	39.13±2.222 (37.3%)[c]↓	76.33±2.160 (48.3%)[c, d]↑

The results are expressed as the Mean±SD of six rats/group; One way ANOVA followed by Tukey's multiple test

a = Results significantly different from Normal group, $P < 0.01$; b = Results significantly different from CCl_4 group, $P < 0.01$

c = Results significantly different from CCl_4 group, $P < 0.05$; d = Results significantly different from EtOH (400 mg/kg), $P < 0.05$

DISCUSSION

The maximum extractable were found in sonicated ethyl acetate fraction (81.25% w/w) of ethanol extract (19.2% w/w) in comparison to the conventional solvent fraction (28.06% w/w) as showed in table 1. Phytochemical investigations revealed the presence of phenolic compounds and flavonoids in the ethyl acetate fraction which are not reported earlier.

The LD_{50} of the seeds was found to be above than 2000 mg/kg. Thus, it would be safe to use this drug as a hepatoprotective. The dose level can be increased to 1000 mg/kg to get more effects *at par* with the silymarin.

The significant ($P < 0.01$) increase in levels of serum AST (60.61±4.654 to 175.8±5.274, ALT (50.11±3.546 to 167.2±3.645) and ALP (163.3±5.399 to 275.40±4.867) confirmed the hepatotoxicity in the group of rats administered with CCl_4 as shown in table 2 (Clawson, 1989; Yadav and Dixit, 2003).

Pretreatment of group of rats with the ethanol extract at dose level of 200 and 400 mg/kg showed significant ($P < 0.05$) hepatoprotection against hepatic injury by restoring the levels of AST (29.25% and 58.0%), ALT (35.5% and 66.1%) and ALP (10.8% and 41.4%) respectively.

Groups of rats pretreated with the ethyl acetate fraction at dose level of 200 and 400 mg/kg showed more significant ($P < 0.05$) improvement in levels of the AST (48.7% and 74.8%), ALT (54.5% and 74.3%) and ALP (31.7% and 51.7%) respectively. The animals pretreated with the silymarin (50 mg/kg) showed highly significant ($P < 0.01$) reduction in rise in the serum enzymes level, *viz*. AST (92.7%), ALT (95.0%) and ALP (74.5%) in comparison to CCl_4 control group.

Table 2 shows the significant ($P < 0.01$) decrease in the levels of TP (6.292±0.518 to 3.270±0.788) and ALB (3.427±0.422 to 2.030±0.159) in CCl_4 control group. Hypoalbuminemia most frequently occurs in the liver disorders. Hence, decline in total proteins content can be deemed as a useful index of the severity of cellular dysfunction in liver disorders. Ethanol extract treated group checked on the fall of the levels of TP by 27.3% (200 mg/kg), 69.4% (400 mg/kg) and on ALB by 18.2% (200 mg/kg) and 47.8% (400 mg/kg). Ethyl acetate fraction showed the significant rise in the level of TP by 48.3% (200 mg/kg), 82.9% (400 mg/kg) and on ALB by 36.6% (200 mg/kg) and 62.0% (400 mg/kg). The decrease in TP observed in CCl_4 control group rats may be associated with the decrease in the number of hepatocytes which in turn, may result into the decreased hepatic capacity to synthesize protein, but the restoration of the level of TP with the pretreatment of the ethanol extract and ethyl acetate fraction confirmed the hepatoprotective nature of *Lawsonia inermis* (Shahjahan *et al.*, 2004).

Group of rats toxicated with CCl_4 (control group) showed the significant ($P < 0.01$) increase in the levels of hepatic MDA (20±2.988 to 50.47±4.088). MDA, a secondary product of lipid peroxidation, is used as an indicator of tissue damage involving a series of chain reactions (Ohkawa *et al.*, 1979). It reacts with thiobarbituric acid, producing red-coloured products. Lipid peroxidation has been implicated in the pathogenesis of increased membrane rigidity, osmotic fragility, reduced erythrocyte survival and perturbations in lipid fluidity. It has been hypothesized that one of the principal cause of CCl_4 induced hepatotoxicity is lipid peroxidation of hepatocyte membranes by free radical derivatives of CCl_4 (Recknagel *et al.*, 1989). The observation of elevated levels of hepatic MDA in CCl_4 control group in the present study is consistent with this hypothesis. Thus, the significant ($P < 0.05$) check on the rise in the level of hepatic MDA in rats treated with the ethanol extract as 6.6% (200 mg/kg) and 24.1% (400 mg/kg) of hepatoprotection; its ethyl acetate fraction group showed 15.8% (200 mg/kg) and 37.3% (400 mg/kg) of hepatoprotection. The group of rats pretreated with silymarin showed highly significant ($P < 0.01$) check on the rise of the MDA with 62.0% hepatoprotection in comparison to the CCl_4 control group (Table 2).

Table 2 shows the reduction of the glutathione level due to the carbon tetrachloride induced hepatotoxicity in rats from 106.20±7.360 to 48.50±4.037. CCl_4 induces fatty liver and cell necrosis (Pencil et al. 1984) and plays a significant role in depletion of GSH (Recknagel *et al.*, 1989). GSH is a major, non-protein thiol in living organisms which performs a key role in coordinating innate antioxidant defence mechanisms. GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects. It is widely known that a deficiency of GSH within living organisms can lead to tissue disorder and injury. The groups of rats pretreated with the ethanol extract and ethyl acetate fraction at dose level of 200 and 400 mg/kg showed increase in GSH level by 8.4%, 24.9% and 12.8%, 48.3% respectively as compared to CCl_4 control group. The rise in GSH level in group pretreated with the ethyl acetate (400 mg/kg) was more significant ($P < 0.05$) in comparison to ethanol extract pretreated group. This may be due to more content of flavonoids (antioxidants) present in ethyl acetate fraction which protects the liver against the attack of the free radicals, peroxides and other toxic compounds.

The ethanol extract and ethyl acetate fraction used in the study preserved the structural integrity of the hepatocellular membrane in a dose dependent manner as evident from the protection provided as compared to the enzyme levels in CCl_4 control group rats. Ethyl acetate fraction at dose level 400 mg/kg showed prominent hepatoprotection in comparison to the ethanol extract pretreated group of rats.

Comparative histopathological study of the liver from different groups of rats corroborated the hepatoprotective efficacy of *Lawsonia inermis* (Figure 1). Various pathological changes like massive fatty changes, gross necrosis and broad infiltration of lymphocytes and Kupffer cells around the central vein and loss of cellular boundaries seen in CCl_4 control group rats were prevented to a moderate extent in silymarin group (50 mg/kg) and the ethyl acetate group (400 mg/kg) (Krajian, 1963).

The results obtained from the present study demonstrated that the ethyl acetate fraction (400 mg/kg) of ethanol extract of the seeds of *Lawsonia inermis* exhibited significant ($P < 0.05$) hepatoprotective effect against CCl_4 induced liver damage may be due to the presence of flavonoids which have hepatoprotective and inhibition of lipid peroxidation properties (Tapas *et al.*, 2008).

In conclusion, the present results demonstrated the ethanol (90%) extract of the seeds of *Lawsonia inermis* have hepatoprotective potential. The ethyl acetate fraction had improved its efficacy. Sonication technique yielded higher percentage of extractables in comparison to conventional techniques. The ethyl acetate fraction contains higher percentage of flavonoids (antioxidants) responsible for hepatoprotective activity. The hepatoprotective activity of ethyl acetate fraction may be due to the inhibition of the production of trichloromethyl peroxy radical (Highly reactive species) when challenged with CCl_4. Further investigation is underway to determine the responsible phytoconstituent/s and to confirm the mechanisms responsible for hepatoprotective activity.

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ABBREVIATIONS

ALB= Albumin; ALT= Alanine transaminase; ALP= Alkaline phosphatase; AST= Aspartate transaminase; CAT= Catalase; CCl_4= Carbon tetrachloride; DTNB= 5, 5'-dithio-bis-2-nitrobenzoic acid; EDTA= Ethylene diamine tetra acetic acid; EtOH= Ethanol; EtOAc= Ethyl acetate; GSH= Reduced glutathione; MDA= Malondialdehyde; TB= Total bilirubin; TBARS= Thiobarbituric acid reactive substances; TP= Total proteins; i.p.= Intraperitoneal; b. wt. = Body weight; p.o.= Per-oral.

REFERENCES

Ahmed S, Rahman A, Alam A, Saleem M, Athar M, Sultana S. Evaluation of the efficacy of *Lawsonia alba* in the alleviation of carbon tetrachloride induced oxidative stress. *J. Ethnopharmacol*, 69, 2000, 157-164.

Ali NAA, Julich WD, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. *J. Ethnopharmacol*, 74, 2001, 173-179.

Chandan BK, Saxena AK, Shukla S, Sharma N, Gupta DK, Suri KA, Suri J, Bhadauria M, Singh B. Hepatoprotective potential of *Aloe barbadensis* Mill. against carbon tetrachloride induced hepatotoxicity. *J. Ethnopharmacol*, 111, 2007, 560-566.

Clawson G. A mechanism of carbon tetrachloride hepatotoxicity. *Pathol. & Immunopathol. Res*, 8, 1989, 104-112.

Doumas BT, Watson WA. BCG method. *Clin. Chem. Acta*, 31, 1971, 87.

Doumas BT. Biuret method. *Clin. Chem*, 21, 1975, 1159.

Ellman GL. Tissue sulphydryl groups. *Arch. Biochem. Biophys*, 82, 1959, 70-77.

Endrini S, Rahmat A, Ismail P, Taufiq-Yap YH. Comparing of the cytotoxicity properties and mechanism of *Lawsonia inermis* and *Strobilanthes crispus* extract against several cancer cell lines. *J. Med. Sci*, 7, 2007, 1098-1102.

Gupta M, Mazumder UK, Kumar TS, Gomathi P, Kumar RS. Antioxidant and hepatoprotective effects of *Bauhinia racemosa* against paracetamol and carbon tetrachloride induced liver damage in rats. *IJPT*, 3, 2007, 12-20.

Harborne JB, Phytochemical methods, 3rd ed., Chapman and Hall, London, 1998, 16-30.

IFCC. Mod. IFCC method for the measurement of catalytic concentration of enzymes. *J. Clin. Chem. Clin. Biochem*, 24, 1998, 481-497.

Iyer MR, Pal SC, Kasture VS, Kasture SB. Effect of *Lawsonia inermis* on memory and behaviour mediated via monoamine neurotransmitters. *Ind. J Pharmacol*, 30, 1998, 181-185.

Jendrassik L, Grof P. Mod. Jendrassik & Grof's method. *Biochem*, 2, 1938, 297.

Kapur V, Pillai KK, Hussian SZ, Balani DK. Hepatoprotective activity of 'Jigrine' on liver damage caused by alcohol, carbon tetrachloride and paracetamol in rats. *Ind. J. Pharmacol*, 26, 1994, 35.

Kind PRH, King EJ. Mod. Kind & King's method. *J. Clin. Path*, 7, 1954, 322.

Krajian AA. Tissue cutting and staining. In: Frankel, S., Reitman, S., (Eds.), Gradwohl's Clinical Laboratory Methods and Diagnosis. The CV. Mosby Co. Saint Louis, USA, 1963, 1639.

Mikhaeil BR, Badria FA, Maatooq GT, Amer MMA. Antioxidant and immunomodulatory constituents of henna leaves. *Z. Naturforschung*, Sec. C. 59, 2004, 468-476.

Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. *Anal Biochem*, 95, 1979, 351-358.

Pencil SD, Brattin WJ, Gelnde EA, Recknagel RL. Carbon tetrachloride dependent inhibition of lipid secretion by isolated hepatocytes: characterization and requirement for bioactivation. *Biochem. Pharmacol*, 33, 1984, 2419-2423.

Recknagel RO, Glonde EA, Dolak JA, Walter RL. Mechanism of carbon tetrachloride toxicity. *Pharmacol. & Therap*, 43, 1989, 139-154.

Sastri BN, The Wealth of India: Raw Materials. 1st ed., 6(L-M), CSIR, New Delhi, 1962, 47-50.

Sengottuvelu S, Duraisamy R, Nandhakumar J, Sivakumar T. Hepatoprotective activity of *Cleome viscosa* against carbon tetrachloride induced hepatotoxity in rats. *Phcog. Mag*, 3, 2007, 120-123.

Shahjahan M, Sabitha KE, Jainu M, Devi CSS. Effect of *Solanum trilobatum* against carbon tetrachloride induced hepatic damage in albino rats. *Ind. J. Med. Res*, 120, 2004, 194-198.

Syamsudin I, Winarno H. The effect of Inai (*Lawsonia inermis*) leaves extract on blood sugar level: an experimental study. *Res. J. Pharmacol*, 2, 2008, 20-23.

Tapas AR, Sakarkar DM, Kakde RB. Flavonoids as nutraceuticals: a review. *Tropical J. Pharma. Res*, 7, 2008, 1089-1099.

Victor J, Navarro MD, John R, Senior MD. Drug related hepatotoxicity. *N. Engl. J. Med*, 354, 2006, 731-739.

Yadav NP, Dixit VK. Hepatoprotective activity of leaves of *Kalanchoe pinnata* Pers. *J. Ethanopharmacol*, 86, 2003, 197-202.