Original Article

Evaluation of *Moringa oleifera* Seeds for Prophylactic and Curative Effects in CCl_4 Induced Rat Liver Injury

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ABSTRACT

The present research work was designed to establish the possible preventive and curative hepatoprotective efficacy on CCl_4 poisoned rat liver toxicity. Fresh mature seeds were shade dried at room temperature, coarse powdered and extracted with 70% hydroalcohol by Soxhlet's extraction method. Thereafter, the extract was concentrated using rotary flash evaporator to obtain semisolid crude extract with the yield of 10.52%. *Moringa oleifera* seeds extract was investigated in graded doses (100, 250 and 500 mg/kg) on CCl_4 mediated liver injury in rats. Antihepatotxic potential was assessed by the estimation of biochemical markers viz. SGPT, SGOT, ALP, ACP and bilirubin (Direct & Total). In addition, liver GSH and MDA levels and histopathological examination were also studied. The dose dependent significant reduction of biochemical markers and bilirubin levels were seen in rats subjected to both pre and post treatments of test extract. Though, the extract at a dose of 100 mg/kg exhibited considerable reduction in concentration of ACP, TB and DB in pre and post treatment modes, but the results were found to be statistically not significant. Hepatic control rats showed significant decrease in hepatic GSH content and elevation of MDA (Malondialdehyde) level compared to normal control group. Pre and post treatment of text extract dose dependently reversed the CCl_4 mediated altered hepatic GSH and MDA levels. The histopathological examination of rat liver sections were supported the hepatoprotective activity of the test extract. The findings of the present investigation suggest that seeds extract of the title plant possesses equally potent prophylactic and curative hepatoprotective efficacy against CCl_4 rendered liver injury in rats.

Keywords: *Moringa oleifera*, CCl_4, hepatoprotective, SGPT, SGOT, ALP, TB and DB.

INTRODUCTION

Hepatic disorders are the most serious ailment and are mainly caused by toxic chemicals (carbon tetrachloride, chemotherapeutic agents, peroxidised oil, excess consumption of alcohol, high doses of paracetamol, etc).[1] Conventional hepatoprotective drugs used for the treatment of such liver diseases are often inadequate and it is needed to dechallenge the offending drug. Towards these pathologies modern medicine does not find any curative treatments.[2] Plant drugs are known to play a vital role in the management of liver diseases.

Hence, searching the safe and potent herbal hepatoprotective remedies has become most fascinating and desired area of research for the pharmacologists. Literature review showed that some of the Indian medicinal plants used traditionally in the management of liver disorders have been scientifically investigated and reported for their measurable hepatoprotective effects against various experimental animal models. However, still more numbers of medicinal plants are needed to be screened for their hepatoprotective efficacy.

Moringa oleifera (family: Moringaceae) commonly known as drumstick is one such plant cultivated for different purposes such as medicine, vegetable, spice, for cooking and cosmetic oil.[3] The leaves, fruits, flowers and immature pods of this tree are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa.[4–6]

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All the parts of the tree are used in folk medicine practices for the treatment of various diseases such as UTI, HIV-AIDS, external sores and ulcers, diabetes, cancer, gastritis, diarrhoea, liver diseases[7] etc. The plant is reported to possess anti-inflammatory,[8] antioxidant,[9] antiulcer,[10] anticancer,[11] antihyperlipidaemic[12] and cardiotonic[13] properties. A study on ethanol and aqueous extracts of whole pods and its parts, i.e. coat, pulp and seed revealed that the blood pressure lowering effect of seed was more pronounced with comparable results in both ethanol and water extracts indicating that the activity is widely distributed[14].

In-vivo hepatoprotective activity of the *Moringa oleifera* leaves,[15] flowers,[16] roots[17] seeds[18] and Pods (fruits)[19] have been already documented in the literature. However, comparative studies on prophylactic (preventive) and curative potential of seeds of the title plant on CCl_4 damaged rat liver has not been investigated so far. Hence, the present investigation was undertaken.

MATERIALS AND METHODS

Plant materials

For this study, the seeds of *Moringa oleifera* were collected from the surrounding gardens of the Harapanahalli, Karnataka, India after the plant material authenticated by Professor K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli, India. A voucher specimen has been deposited at the museum of the college.

Preparation of extract

Fresh mature seeds were shade dried at room temperature, coarse powdered and extracted with 70% hydro-alcohol by Soxhlet's extraction method. Thereafter, the extract was concentrated using rotary flash evaporator to obtain semisolid crude extract. The percentage yield of the extract was found to be 10.52. The extract was stored in airtight container in refrigerator below 10°C. Desired concentration of stock solution was prepared using distilled water for the following studies.

1. Preliminary phytochemical investigation.
2. Acute toxicity study in mice.
3. Evaluation of hepatoprotective activity against CCl_4 induced liver injury in rats.

Preliminary phytochemical screening[20]

Preliminary phytochemical tests were conducted on test extract to detect the presence of phytochemicals by following the standard methods described in the Pharmacognosy text book of Trease and Evans.

Experimental animals

Male albino Wistar rats (150–200 g) and female albino Swiss mice (20–25 g) were used in the experiments. They were procured from Sri Venkateshwar enterprises, 4304, 13th main 2nd cross, Subramanya nagar, Bangalore-21 (237/CPCSEA), India. After randomization into various groups and before initiation of experiment, the animals were acclimatized for a period of 10 days. Animals were housed in polypropylene cages and maintained under standard environmental conditions such as temperature (26 ± 2°C), relative humidity (45–55%) and 12 hr. dark/light cycle. The animals were fed with rodent pellet diet and water *ad libitum*. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC) before commencement of experiment. The Ref. No. SCSCP/665/2008-09.

Determination of acute toxicity (LD_{50})[21]

The acute toxicity of test extract was determined in mice weighing between 20–25 g following fixed dose method of CPCSEA, OECD (Organization for Economic Cooperation and Development), guideline No. 420; (Annexure-2d). $1/25^{th}$, $1/10^{th}$ and $1/5^{th}$ LD_{50} cut-off value of the extract were selected as screening doses for the hepatoprotective study.

Evaluation of hepatoprotective activity on CCl_4 rendered hepatic damage in rats[22]

Albino rats of Wistar strain weighing 150–200 g were allocated to 08 groups of six each as shown below.

Group 1: Normal control - Untreated.

Group 2: CCl_4 control - Injected with fresh mixture of equal volume of CCl_4 and liquid paraffin by 3 i.p. injections at a doses of 2 ml/kg body weight.

Pre treatment of *Moringa oleifera* seeds extract (Prophylactic study)

Group 3: 100 mg/kg
Group 4: 250 mg/kg
Group 5: 500 mg/kg

Groups 3, 4 and 5 were given orally for 14 consecutive days and injected intraperitonially CCl_4 (2 ml/kg in equal volume of liquid paraffin) **on days 12, 13 and 14.**

Post treatment *Moringa oleifera* seeds extract (Curative study)

Group 6: 100 mg/kg
Group 7: 250 mg/kg
Group 8: 500 mg/kg

Groups 6, 7 and 8 were given orally for 14 consecutive days and injected intraperitonially CCl_4 **on days 1, 2 and 3.**

During the period of treatment the rats were maintained under normal diet and water. Twenty four hours after the last treatment i.e. on 15^{th} day, the blood samples were collected by puncturing the retro orbital

plexus under the influence of light ether anesthesia and allowed to coagulate for 30 min at 37°C. Plasma was separated by centrifugation at 3000 rpm for 15 min and used for estimation of biochemical parameters such as Serum glutamic-pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), Alkaline phosphatase (ALP), Acid phosphatase (ACP) and bilirubin (Total and Direct) using ready Erba Diagnostics Mannheim GmbH – Germany kits by STAR 21 PLUS Semi autoanalyser. Then all the rats were euthanized and their liver tissues were excised, rinsed in ice cold normal saline to remove adhered blood. A section from the median lobe was preserved in 10% formalin for histopathological studies. Rest of the liver tissues were subjected to homogenization and the resulted liver homogenate was used for the estimation of GSH and LPO following literature reported methods.[23,24]

Statistical analysis

The results generated from the present investigation were subjected to statistical analysis using ANOVA followed by Turkey Kramer Multiple Comparison Test. Results are expressed as Mean ± SEM.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical evaluation of the *Moringa oleifera* 70% hydro alcoholic seed extract revealed the presence of flavonoids, tannins, glycosides and carbohydrates.

Determination of acute toxicity (LD_{50})

In an acute toxicity studies, test extract of title plant did not cause any mortality of the animals at dose of 2000 mg/kg. Hence, 2500 mg/kg was fixed as LD_{50} cut-off value as per fixed dose method of CPCSEA.

The doses chosen for the evaluation of hepatoprotective activity of the test extract were:

100 mg/kg - $1/25^{th}$ dose of 2500 mg/kg b.w.

250 mg/kg - $1/10^{th}$ dose of 2500 mg/kg b.w.

500 mg/kg - $1/5^{th}$ dose of 2500 mg/kg b.w.

Effect of *Moringa oleifera* seeds extract on biochemical parameters in CCl_4 damaged rat liver

The CCl_4 control rats exhibited significant elevation of serum marker enzymes SGPT, SGOT, ALP, ACP and bilirubin (Total & Direct) levels indicating hepatocellular damage when compared with normal control group. The dose dependent significant reduced concentrations of SGPT, SGOT, ALP, ACP, total and direct bilirubin were monitored in rats subjected to both pre and post treatment of test extract. Though, the extract at dose of 100 mg/kg exhibited considerable reduction in concentration of ACP, total and direct bilirubin in pre and post treatment modes, but the results were found to be statistically not significant. The results are tabulated in Table 1.

Effect of test extract on liver GSH and LPO levels

In CCl_4 poisoned rats a significant decrease in GSH level was observed compared to normal control group. Pre and post treatment with the test extract significantly attenuated CCl_4 induced decrease of GSH content in dose dependent manner compared to CCl_4 intoxicant group.

The effect of test extract on CCl_4 mediated LPO was examined through monitoring the levels of MDA (Malondialdehyde). The extract of title plant on pre and post treatments dose dependently reversed the CCl_4 intoxicated elevation of hepatic MDA level. Though, there was considerable decrease in LPO content monitored in rats with pre and post treatments of test extract at a dose of 100 mg/kg, but the results were found to be not significant statistically. The results are given in Table 2.

Table 1: Preventive and Curative Effects of *Moringa oleifera* Seeds Extract on Biochemical Markers

Groups	SGPT	SGOT	ALP	ACP	TB	DB
Normal control	117.98 ± 4.13	145.1 ± 4.67	291.25 ± 1.33	30.45 ± 1.27	0.370 ± 0.007	0.283 ± 0.012
CCl_4 control	250.00 ± 2.33	350.0 ± 6.16	560.80 ± 6.60	55.60 ± 1.22	0.639 ± 0.004	0.551 ± 0.007
Preventive						
100 mg/kg	230.30 ± 3.40*	320.30 ± 4.57**	480.42 ± 5.20***	43.01 ± 2.80^{ns}	0.501 ± 0.007^{ns}	0.478 ± 0.002^{ns}
250 mg/kg	170.28 ± 5.80***	270.80 ± 3.80***	345.43 ± 2.80***	39.05 ± 2.83*	0.463 ± 0.009*	0.353 ± 0.07**
500 mg/kg	125.87 ± 4.83***	157.18 ± 5.81***	310.14 ± 3.85***	33.07 ± 4.01***	0.390 ± 0.031**	0.297 ± 0.03***
Curative						
100 mg/kg	227.60 ± 5.70*	317.30 ± 4.30***	489.70 ± 4.90***	42.09 ± 3.10^{ns}	0.498 ± 0.007^{ns}	0.473 ± 0.003^{ns}
250 mg/kg	173.53 ± 3.73***	268.30 ± 5.78***	349.60 ± 2.30***	38.90 ± 3.10*	0.458 ± 0.021*	0.347 ± 0.03**
500 mg/kg	127.12 ± 3.89***	153.43 ± 4.90***	315.57 ± 3.80***	33.07 ± 2.07***	0.380 ± 0.081***	0.293 ± 0.03***

Results are Mean ± SE, n = 6, *p < 0.05, **p < 0.01 and ***p < 0.001 compared to CCl_4 control.

Table 2: Preventive and Curative Effects of *Moringa oleifera* Seeds Extract on GSH and LPO Levels

	GSH		LPO	
Groups	**Absorbance mean ± SEM**	**% increase**	**Absorbance mean ± SEM**	**% inhibition**
Normal control	0.783 ± 0.057	–	0.225 ± 0.008	–
CCl_4 control	0.251 ± 0.032	–	0.527 ± 0.112	–
Preventive				
100 mg/kg	0.462 ± 0.033*	84.06	0.388 ± 0.020*	32.16
250 mg/kg	0.609 ± 0.039***	142.62	0.309 ± 0.018***	45.97
500 mg/kg	0.670 ± 0.053***	166.93	0.279 ± 0.013***	51.22
Curative				
100 mg/kg	0.470 ± 0.019*	70.15	0.392 ± 0.019*	31.46
250 mg/kg	0.613 ± 0.029***	144.22	0.312 ± 0.020***	45.45
500 mg/kg	0.668 ± 0.052***	166.13	0.285 ± 0.017***	50.17

Results are Mean ± SE, n = 6, *p < 0.05, **p < 0.01 and ***p < 0.001 compared to CCl_4 control.

Effect of *Moringa oleifera* seeds extract on histopathological profile

Histopathological profile of liver from CCL_4 (Hepatic control group) intoxicated rats reveals hepatic globular architecture disrupted, hepatic cells has shown various degree of fatty degeneration like ballooning of hepatocytes, fatty cyst, infiltration of lymphocytes, proliferation of kuffer cells and congestion of liver sinusoids. Pre and post treatment of test extract at the dose of 500 mg/kg showed a significant improvement of the hepatic architecture (Fig. a–d) and areas of Kupper cell proliferation and sinusoid appeared normal on contrary with 100 and 250 mg/kg doses of the extract.

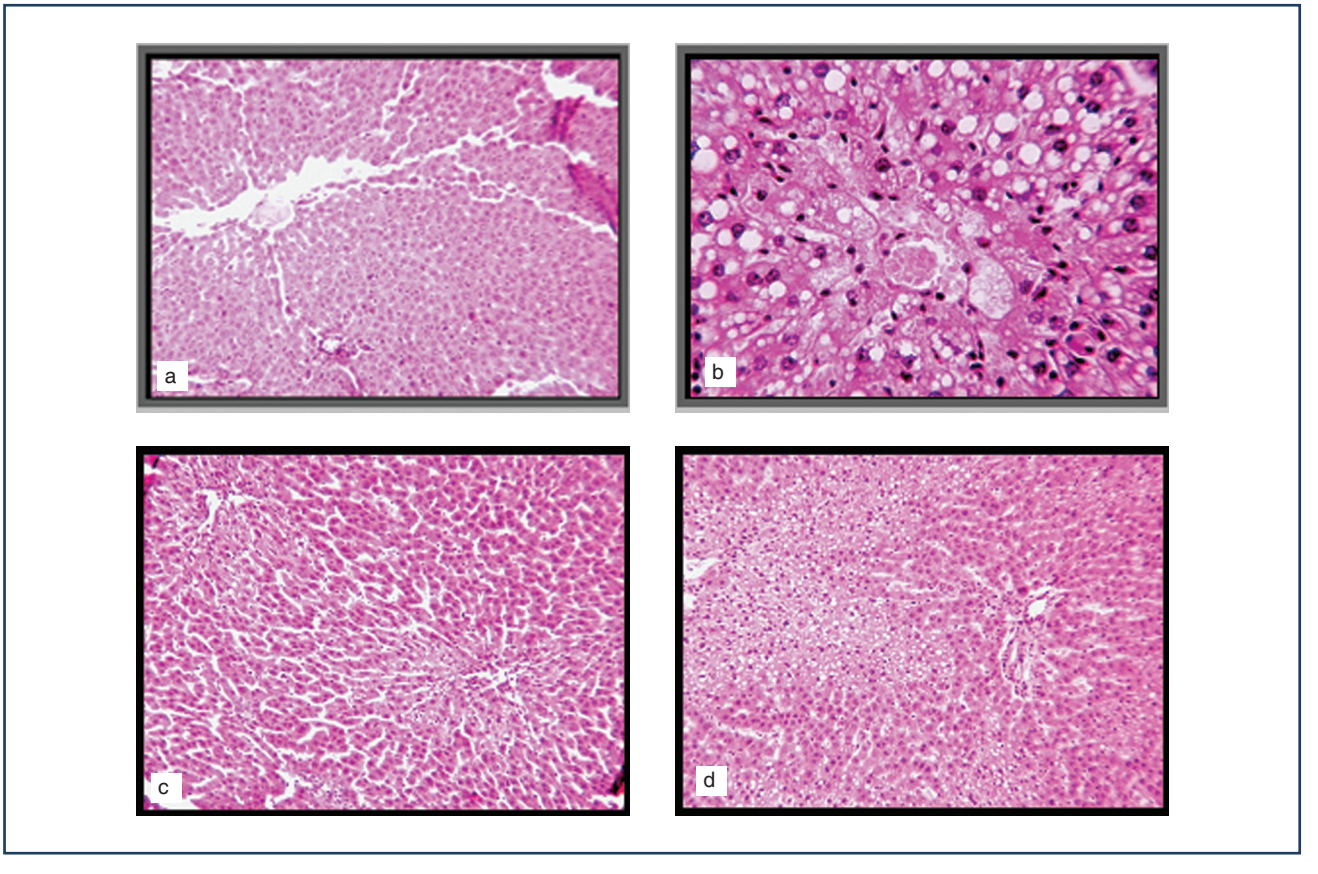

Figure 1: Showing histopathological observations of rat liver sections
a. Normal control
b. Hepatic control
c. Pretreatment at dose of 500 mg/kg
d. Posttreatment at dose of 500 mg/kg

DISCUSSION

The aim of current investigation was to study the prophylactic (preventive) and curative effects of *Moringa oleifera* seeds extract on CCl_4 poisoned liver injury in rats. Liver toxicity induced by CCl_4 is perhaps widely used experimental model for the screening of hepatoprotective agent.[25] Several mechanisms underlying this toxicity have been suggested. CCl_4 the inactive metabolite is biotransformed by cytochrome P – 450 systems to produce the trichloromethyl free radical (CCl_3•) that causes lipid peroxidation and there by produce liver damage.[26–28]

The dose dependent significant reduction of CCl_4 rendered elevated plasma concentrations of SGPT, SGOT, ALP, ACP, total and direct bilirubin in rats pre and post treated with 70% hydro-alcoholic extract of *Moringa oleifera* seeds demonstrated their ability to restore the normal functional status of the poisoned liver, and also to protect against subsequent CCl_4 liver injury.

The development of hepatotoxicity induced by CCl_4 challenge was exacerbated following the depletion of glutathione. Therefore, in the current study glutathione level was measured to observe the preventive and curative effects of *Moringa oleifera* seeds in experimental animals. The results presented in the Table 2 clearly demonstred that CCl_4 intoxication has significantly reduce the glutathione level compared to normal control animals. Rats on pre and post treatment with *Moringa oleifera* seeds extract (100, 250 and 500 mg/kg, doses) have clearly restored the levels of glutathione significantly in a dose related manner.

The CCl_4 damaged liver toxicity was associated with marked increase in liver MDA level. The MDA elevation has been well accepted reliable marker of lipid peroxidation. MDA elevation is a result of oxidative stress demonstrated here through the decreased liver GSH content. Hence, in the present study MDA level was also estimated to evaluate prophylactic and curative properties of test extract. From the Table 2, it was clearly indicated that CCl_4 intoxicated rats showed significant increase in the MDA level compared to normal control group. Rats on pre and post treatment with *Moringa oleifera* seeds extract (100, 250 and 500 mg/kg, doses) have significantly dose dependently decrease the MDA level.

The findings of the present investigation were also consistent with previous reports, where different parts of *Moringa oleifera* (but only pretreatment mode) shown to protect liver from hepatoxicity caused by diclofenac,[18] ethanol[29] and antitubercular drugs.[30]

The mechanism by which *Moringa oleifera* seeds extract exhibited hepatoprotective activity is not clear from the present study. However, the important factor in the hepatoprotective property of any drug is the ability of its constituents to inhibit the aromatase activity of cytochrome P – 450, thereby favoring liver regeneration.[23] On this basis, it is suggested that flavonoids content in the test extract (evident by Preliminary phytochemical screening) could be the reason for contributing hepatoprotective ability through inhibition of cytochrome P – 450 aromatase.[31]

CONCLUSION

In conclusion, the findings of the present study suggest that seeds extract of the *Moringa oleifera* possesses equally potent prophylactic and curative hepatoprotective efficacy against CCl_4 rendered liver injury in rats.

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