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Hepatoprotective Studies on Aerial Parts of *Moringa oleifera* Lam. on Carbon tetrachloride induced liver cell damage in albino rats

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Abstract

The extracts from leaves, flower and isolated fraction of *Moringa oleifera* Lam. were evaluated for hepatoprotective activity in rats with liver damage induced by Carbon tetra chloride. The extract at an oral dose of 250 mg/kg exhibited a significant protective effect by lowering the serum levels of bilirubin, glutamate pyruvate transferase (SGPT), glutamate oxaloacetate transferase (SGOT), alkaline transferase and lysosomal enzymes. These biochemical observations were supplemented by histopathological examination of liver sections. The activity of extract was also comparable to that of silymarin, a known hepatoprotective agent.

Keywords: *Moringa oleifera*, SGPT, SGOT, ALP, Bilirubin, Carbon tetrachloride, Silymarin.

Introduction

Liver is the largest organ in the vertebrate body and the site for intense metabolism. Liver diseases remain one of the serious health problems and the Indian traditional system of medicine, especially Ayurveda have put forward a number of medicinal plants and their formulations for liver disorders. In this modern age it is very important to provide scientific proof to justify the various medicinal uses of herbs. Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost. However, we are not aware of a satisfactory remedy for serious liver diseases and search for effective and safe drugs for liver disorders continues to be an area of interest. *Moringa oleifera Lam.* (family Moringaceae) is a small genus of quick-growing trees distributed in India, Arabia, Asia Minor and Africa. This tree is indigenous to northwest India. It is widely cultivated and naturalized in tropical Africa, tropical America, Sri Lanka, Mexico, Malabar, Malaysia and the Philippine Islands. Two species are recorded from India of which one *Moringa oleifera* is widely cultivated in the tropics for its edible fruits. It is also known by different name e.g

Moringa pterygosperma Gaertn, Drum stick tree, Horse Radish tree, Shobhanjana(Sans.), Mungna, Sainjna, Shajna (Hindi), Sainjana, Soanjana (Punjabi), sigru, Moringa (Malayalam) [1]. It is a short, slender, deciduous, perennial tree, upto about 10 m tall having brittle branches, with corky bark. Leaves are feathery, pale green, compound, tripinnate, 30–60 cm long, with many small leaflets, 1.3–2 cm long and 0.6–0.3 cm wide, lateral ones somewhat elliptic, terminal one obovate and slightly larger than the lateral ones; flowers are fragrant, white or creamy-white, 2.5 cm in diameter, borne in sprays, with 5 yellow stamens at the top of the flower, pods pendulous, brown, triangular, splitting lengthwise into 3 parts when dry, 22.5-50 cm or more in length containing about 20 seeds embedded in the pith. Pods taper at both ends, are 9-ribbed, and seeds are dark brown, with 3 papery wings. The main root is thick. Fruit or other parts of plant are usually harvested as desired, according to some authors, but in India, fruiting may peak between March and April and again in September and October. Seeds are gathered in March and April and oil expressed [1-2].

All parts of the tree are considered medicinal and used in treatment of ascites, rheumatism, and venomous bites and as cardiac and circulatory stimulants. The root of young tree and also root bark are rubefacient and vesicant. The leaves are rich in Vit. A and C and are considered useful in scurvy and catarrhal affections; they are also used as emetic. A paste of leaves is used as an external application for wounds. Flowers are used as tonic, diuretic and cholagogue. Seeds are considered as antipyretic acrid and bitter. Seed oil is used as anti-inflammatory in rheumatism and gout [3]. The flowers, leaves, and roots are used in folk remedies for tumors and the seed for abdominal tumors. The root decoction is used in Nicaragua for dropsy. Root juice is applied externally as rubefacient or counter-irritant. Leaves are applied as poultice to sores, rubbed on the temples for headaches, and said to have purgative properties. Bark, leaves and roots are acrid and pungent, and are taken to promote digestion. Oil is somewhat dangerous if taken internally, but is applied externally for skin diseases. This plant is also reported for antidiabetic activity [4].

Materials and Methods

Plant material

Moringa oleifera leaves and flowers were collected from local fields of Lucknow, Uttar Pradesh, India. A voucher specimen was deposited at taxonomy lab, ethnopharmacognosy division, National Botanical Research Institute (NBRI), Lucknow, India for future reference (No: NBRI/CIF/Re./08/2008/32).

Preparation of extract

Dried leaf powder (1 kg) was extracted with chloroform and methanol and dried flower powder was extracted with methanol by continuous hot percolation (with soxhlat apparatus) for 8 hrs. All the extracts were concentrated and tested for qualitative phytoconnstituents and indicated the presence of steroids, alkaloids and Flavonoids [5].

Animals

Male albino rats weighing 150-200 gm were used for study of anti-inflammatory activity and male albino rats weighing 150-200 gm were used for study of hepatoprotective activity.

All the animals were kept under standard environmental condition. Animals were given standard diet of Hindustan Liver Limited and water ad libitum. All procedures compiled with the norms of animal ethics committee (approval no. BBDNITM/IAEC/clear/12/2008).

Hepatoprotective effect against CCl4-induced hepatotoxicity in rats

The animals weighing 150-200gm were taken for study. They were divided in seven groups having six rats in each. Group I served as control, given mixture of 2.5% DMSO and 2.5% Tween-80 in water. Group II served as Diseased and given carbon tetra chloride (0.5ml/kg body weight i.p.). Group III served as reference and received silymarin 50 mg/kg body weight. Groups IV, V, VI, and VII were given extracts and fractions (250 mg/kg body weight) in mixture of 2.5% DMSO and 2.5% Tween-80 in water orally. For this study the animals were kept on fasting for 16 hrs prior to induce disease. The carbon tetra chloride was given for 5 consecutive days, after that, the extracts were given for 7 consecutive days [6]. After 24 hrs last dose given, the blood was withdrawn and serum was separated out and used for estimation of serum enzymes along with lysosomal enzymes. The liver was immediately removed and was processed for histological studies [Table I].

Table: I Effect of extracts and fractions on carbon tetra chloride induced hepatocellular damage.

S no	Test group	Dose (mg/kg body wt.)	SGPT (IU/L)	SGOT (IU/L)	Serum ALP (IU/L)	Serum bilirubin (mg/dl)
1	Group I	0	49.83±3.77	127.3±5.48	191.84±8.28	0.59±0.02
2	Group II	0	219.33±12.3	343.5±6.68	481.1±11.77	1.72±0.06
3	Group III	50	84.66±3.52***	136.16±7.22***	208.6±7.57***	0.75±0.03***
4	Group IV	250	113.83±3.43***	140.83±4.08***	142.5±9.23***	0.95±0.05***
5	Group V	250	123.16±3.51***	154.17±4.51***	267.67±11.01***	1.13±0.05**
6	Group VI	150	146.17±3.47**	242.67±5.28**	282.2±11.39***	1.02±0.05**
7	Group VII	150	112.42±4.29***	138.40±5.22***	145.67±4.70***	0.91±0.1***

N= 6 Animals in each group; Values are expressed as Mean±SEM; *P<0.05; **P<0.01; ***P<0.001 when compared with disease control; Group I= Normal control, Group II= Disease control, Group III= diseased animals treated with reference (Silymarin), Group IV= diseased animals treated with methanol (leaf extract), Group V= diseased animals treated with methanol (flower extract), Group VI= diseased animals treated with fraction SF (C 1-3), Group VII= diseased animals treated with fraction CF (M 1-4)

Histopathological studies:

The tissues of liver were fixed in 10% formalin and embedded in paraffin wax. Sections of 4-5 microns thickness were made using rotary microtome and stained with haematoxylin-eosin and histological observations were made under light microscope.

Lysosomal enzymes estimation:

The activity of lysosomal enzymes was investigated in blood serum. The blood withdrawn was centrifuged for 10 minutes at 2000 r.p.m. and separated plasma was taken for estimation of cathepsin which corresponds to lysosomal enzyme activity [7].

In the reaction, the lysosomal enzymes particularly cathepsin present in the blood of various animal groups acts on hemoglobin and results in to free tyrosine whose absorbance is measured at 620 nm after developing with Follin's reagent. For animal groups in which tyrosine absorbance readings are higher indicate higher level of cathepsin indicating higher level of lysosomal enzyme activity. Animals treated with reference or therapeutically active fractions should show less tyrosine readings and lysosomal enzyme activity [Table II].

Table: II Lysosomal enzymes inhibition

S. No.	Test material	Absorbance (λmax 620 nm)	% inhibition
1	Group I	0.096	-
2	Group II	0.778	-
3	Group III	0.129	83.41***
4	Group IV	0.170	78.14***
5	Group V	0.208	73.26**
6	Group VI	0.577	25.83
7	Group VII	0.193	75.19***

N= 6 Animals in each group; Values are expressed as Mean±SEM; *P<0.05; **P<0.01; ***P<0.001 when compared with disease control; Group I= Normal control, Group II= Disease control, Group III= diseased animals treated with reference (Silymarin), Group IV= diseased animals treated with methanol (leaf extract), Group V= diseased animals treated with methanol (flower extract), Group VI= diseased animals treated with fraction SF (C 1-3), Group VII= diseased animals treated with fraction CF (M 1-4).

Lysosomal inhibiting activity was expressed as percentage inhibition and estimated by following formula reported in the Reference.

$$\% \text{ Inhibition of lysosomal enzymes} = \frac{A_{(\text{disease control})} - A_{620}}{A_{(\text{disease control})}} \times 100$$

Statistical analysis

The results are expressed as means ± S.D. The difference between experimental groups were compared by one-way ANOVA (Newman's Keuls test) and were considered statistically significant when $p < 0.05$.

Results

The methanolic extract of leaves of *Moringa oleifira* and methanolic extract of flowers, both showed significant activity (P<0.001) and (P<0.01 to 0.001) respectively, against CCl_4 induced increases in these enzymes. The fraction M 1-4 and C 1-3 from methanolic extract of leaves also showed some activities (P<0.001) for CF (M 1-4) and (P<0.01 to 0.001) for SF (C 1-3). The SF fraction showed better reduction in SGOT and ALP than reduction of other enzyme levels [Fig I].

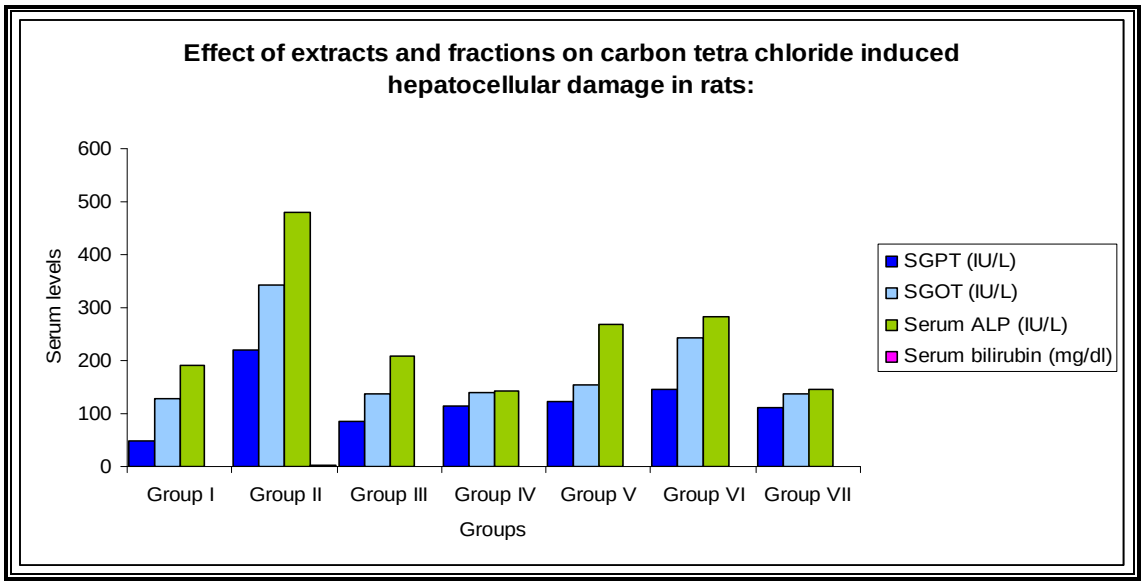

Fig: I Graph showing Hepatoprotective effect against CCl4-induced hepatotoxicity in rats

CCl_4 induced hepatocyte damage also causes damage to the lysosomal membrane causing liberation of degradative enzymes like acid phosphatase, cathepsin etc. followed by cell destruction[8].

The methanolic extract of leaves of *Moringa oleifera* and the methanolic extract of flowers of *Moringa oleifera* showed the significant activities ($P<0.001$) and ($P<0.01$) respectively. The fractions M 1-4 and C 1-3 from methanolic extract of leaves showed activities against lysosomal enzyme level by ($P<0.001$) and ($P<0.05$) respectively [Fig II].

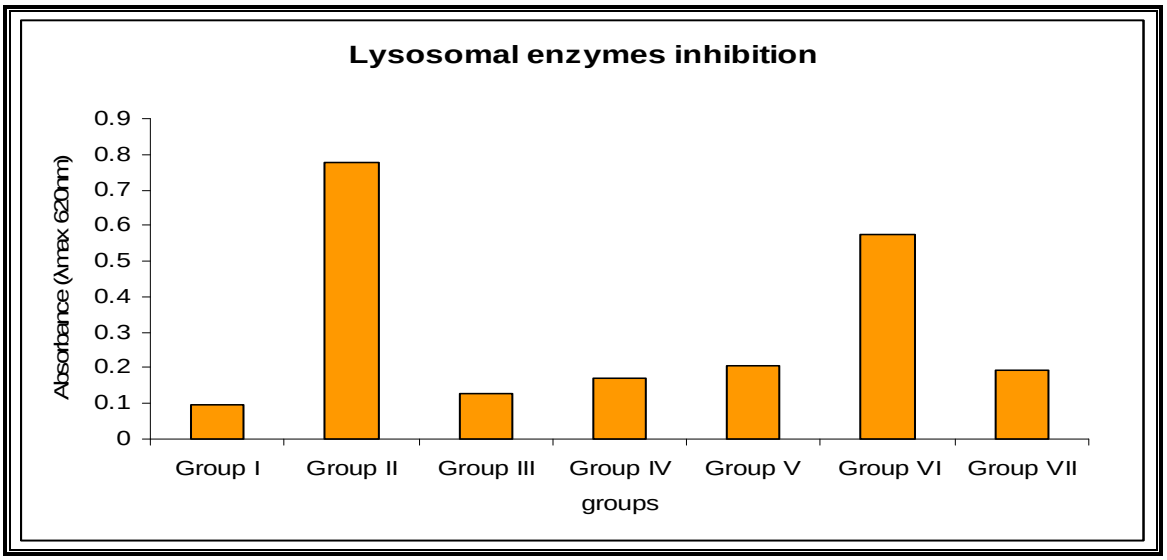

Fig: II Graph showing Lysosomal enzymes inhibition of various extracts and fractions

Carbon tetra chloride induced histological changes:

CCl_4 induced hepatic degeneration causes wide spread cellular damage disturbing the compact cellular arrangement of liver cells [Fig III].

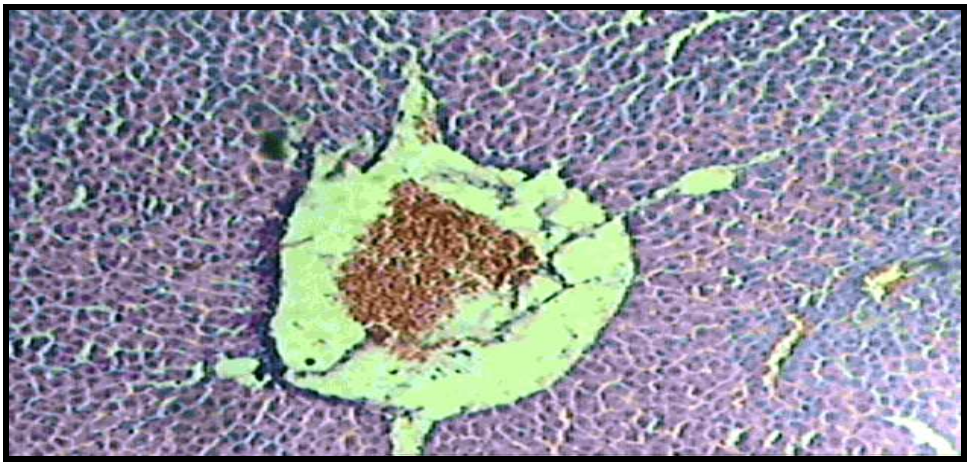

Fig: III Liver histopathological section- Disease control group

Methanolic extract of leaves and CF fraction (M 1-4) and SF fraction (C 1-3) showed considerable normalization of the cellular degeneration [Fig: IV, V, VI, and VII].

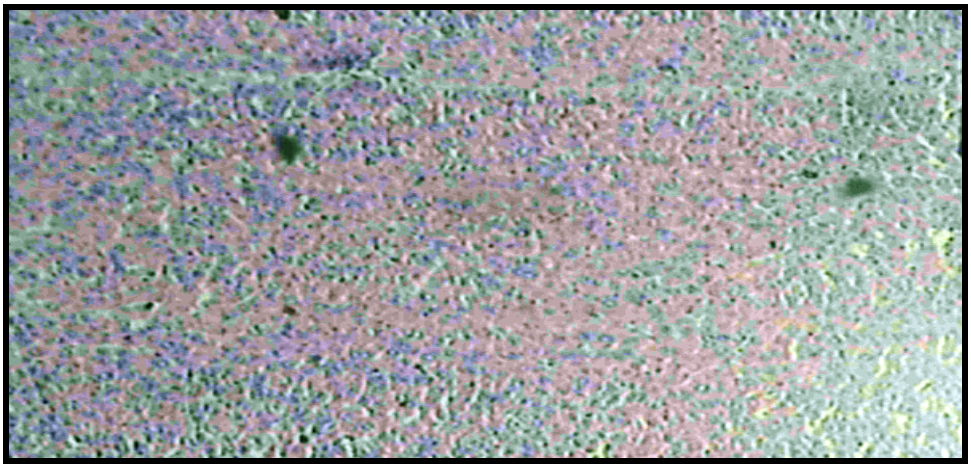

Fig:IV Liver histopathological section-Methanolic leaves extract treated group

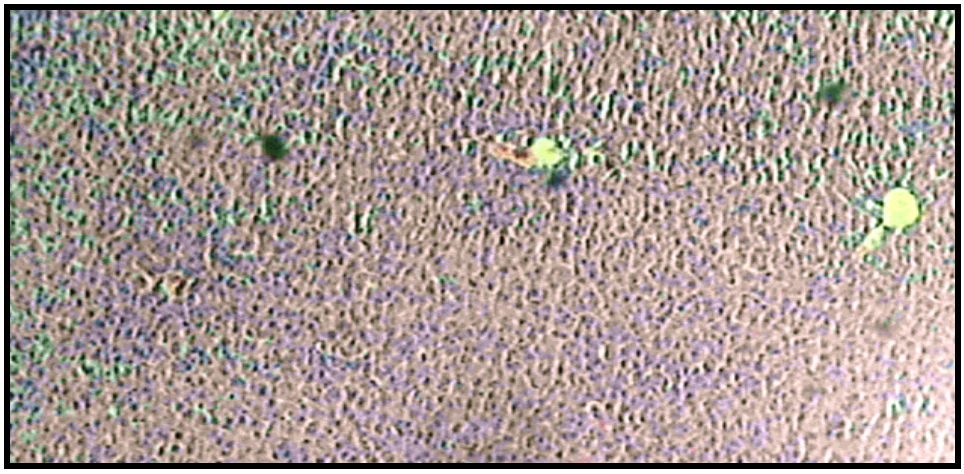

Fig: V Liver histopathological section- Methanolic flower extract treated group

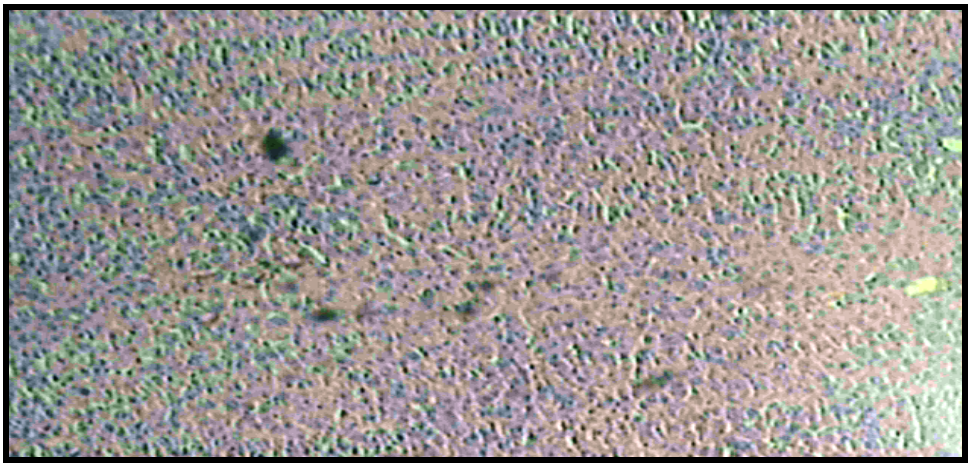

Fig: VI Liver histopathological section- Steroidal fraction SF treated group

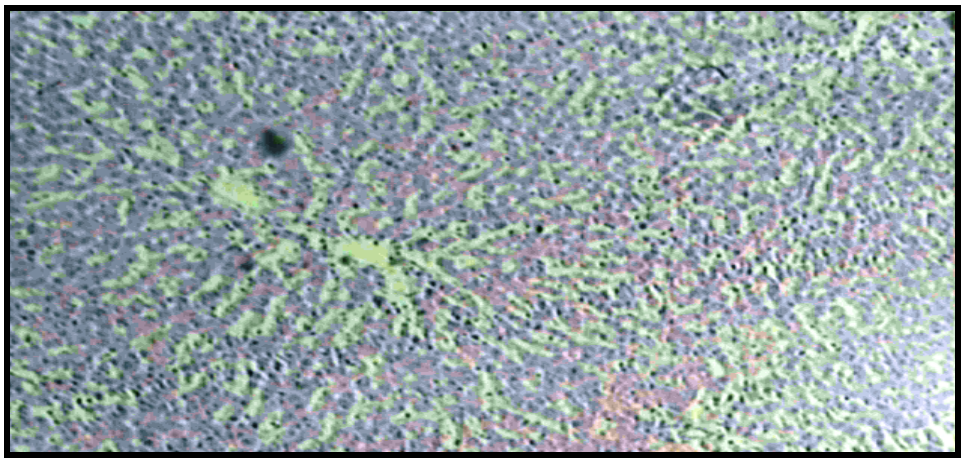

Fig: VII Liver histopathological section- Coumarin fraction CF treated group

Discussion

Carbon tetra chloride (CCl_4) induced hepatic damage causes leakage of SGOT, SGPT, Serum ALP, and serum bilirubin etc. It also causes changes in the cell membrane ratios of cholesterol-phospholipids and sphingomyelins-phosphatidyl cholin [9]. These changes lead to large scale morphological damages in liver hepatocytes.

The activity of the extract and fractions may thus be because of stabilization of hepatocyte membrane and thus reduction in leakage of SGOT, SGPT, albumin etc. in to the plasma.

Thus the hepatoprotective activities of extract and some of the fractions may be because of the restabilization of lysosomal membrane. Lysosomal enzymes if allowed to circulate within plasma causes degradation in the cell wall and inner structure. Substances which inhibit release of lysosomal enzymes and stabilize lysosomal membranes, prevent wide spread damage to cellular and tissue structures which normally accompanies acute or chronic inflammatory conditions which show presence of large number of WBCs and activity of lysosomal enzymes.

Conclusion

The detailed investigation regarding hepatoprotective activity and the constituents responsible for these activities have not been reported and therefore an effort was done to isolate various constituent fractions from leaves and flowers and carryout pharmacological investigation.

Methanolic extract of both leaves and flowers of plant *Moringa oleifera* showed significant hepatoprotective activity as investigated by using various models. The other solvent extracts of the leaves did not show significant hepatoprotective activity. The coumarin fraction of the methanolic extract of leaves showed an overall superior hepatoprotective activity as compared to the steroidal fraction from the leaves as investigated by various models.

The coumarin fraction also showed significant hepatoprotective activity by normalizing changes in biochemical hepatic enzymes produced by carbon tetrachloride and also normalized levels of lysosomal enzyme in blood. Thus, the hepatoprotective action may also be because of its capacity to inhibit inflammatory mediators and prevent cellular injury. Thus the coumarin fraction needs to be further worked upon for commercial extraction and verified for dose response relationship for its hepatoprotective activity.

Further studies need to be carryout to isolate compounds in appreciable quantities and subjected to detailed dose response relationship studies and preclinical toxicity studies as important lead compound for further QSAR study and clinical trials.

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