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EXPLORATION OF HEPATOPROTECTIVE ACTIVITY OF AQUEOUS EXTRACT OF *SOLANUM NIGRUM* - AN EXPERIMENTAL STUDY

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ABSTRACT

Introduction: *Solanum nigrum* (*S. nigrum*) has been shown to be hepatoprotective by Ayurvedic physicians but has not been scientifically evaluated so far. So, the present study was undertaken to explore the hepatoprotective activity of *S. nigrum* against experimentally induced hepatotoxicity in albino rats.

Aim of the study: To explore the hepatoprotective activity of *S. nigrum*.

Materials and Methods: Albino Wistar rats weighing 150-200g of either sex were divided into six groups of six animals each. Group I was given normal saline (PO), group II Carbon tetrachloride (CCl₄) (IP), group III Liv. 52 syrup for twenty days followed by carbon tetrachloride, group IV, V & VI received aqueous extract of *Solanum nigrum* (2ml/100g/day twice daily) orally for 10, 20 & 30 days respectively followed by CCl₄. Blood was collected from anaesthetized animals & liver was dissected out. Alanine transaminase (ALT), alkaline phosphatase (ALP) & Total bilirubin were estimated and liver was subjected to histopathological examination.

Results: ALT, ALP & Total bilirubin levels were significantly increased in CCl₄ treated group but *S. nigrum* displayed significant limitation of rise in these parameters in group IV, V & VI. This hepatoprotection was also reflected in histological changes.

Conclusion: It can be concluded from the present study that *S. nigrum* extract is a potent hepatoprotective agent. It is assumed that this hepatoprotective effect of *S. nigrum* may be due to several reasons such as antioxidant, free radical scavenger and hepatic regeneration.

INTRODUCTION: Liver is one of the largest organs in human body and the chief site for intense metabolism and excretion. So it has a important role in the maintenance, performance and regulation of homeostasis in the body. It is involved in almost all the biochemical pathways related to growth, to fight

against diseases, for nutrient supply, energy provision and reproduction¹. Thus, it is crucial to maintain a healthy liver for overall health and well being. But liver is continuously and variedly exposed to environmental toxins and abused by poor drug habits and alcohol, which can eventually lead to various liver disorders,

generally presenting as a distinct patterns of diseases such as hepatocellular, cholestatic (obstructive), or mixed type of liver disorders.

Almost all types of liver injuries may lead to hepatic failure and ultimately death. Thus liver diseases are one of the most fatal diseases in the world today. Till date available modern drugs have not been able to come up with a satisfactory answer for liver disorders².

In the absence of reliable liver protective drugs in allopathic system of medicine, many herbs have been claimed to play an important role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional system of medicine in India. Liv.52 is such a herbal formulation of The Himalaya Drug Company India. It is a mixture of extracts of 7 herbs and is used for the treatment of various liver diseases. A number of research articles have been published in its favor including experimental as well as clinical studies³.

S. nigrum is a plant which belongs to family solanaceae and has been shown to be hepatoprotective by Ayurvedic physicians. However, the literature reviews indicate that the hepatoprotective activity of *S. nigrum* has not been scientifically evaluated so far.

Therefore, the present study is intended to explore hepatoprotective activity of an aqueous extract of *S. nigrum* in experimentally induced hepatotoxicity in albino rats.

MATERIALS AND METHODS:

Preparation of Plant Extract: The *S. nigrum* plant was obtained from the local market of Meerut, UP (India) in August 2010. The plants were authenticated by the Botany Department of CCS University, Meerut, UP (India). After collection of the required quantity, it was carefully segregated, washed and dried in shade. The whole dried plants and its fruit were separately powdered mechanically with a herb grinder. The powder of whole plant and fruit were mixed in equal ratio manually. The prepared powder was kept in dry, clean, airtight glass jar and stored at 4°C until used. 100

g of the prepared powder was macerated in 500 ml of distilled water for 24 h.

It was then filtered through a 1mm mesh sieve and the filtrate was dried in Petri dishes and concentrated to a dark green residue by heating at 40°C, till complete evaporation of water was achieved.

Animals: Albino rats (Wistar strain) of either sex and weighing 150-200g were obtained from authorized animal house (Jamia Hamdard, Delhi). The animals were housed in cages under controlled conditions of temperature (25°C) and alternating 12 hour cycle of light and darkness. The animals had free access to standard rat pellet diet (Lipton India Ltd.) and tap water *ad lib*. After one week of acclimatization, the animals were considered suitable for study.

Study were reviewed and approved by the Institutional Animal and Ethics Committee of L.L.R.M. Medical College, Meerut, India, and were in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Study Design: These animals were divided into six groups of six animals each.

Group-I: This group was given normal saline 2ml/100g/day per orally in addition to the standard rat pellet diet and tap water.

Group-II: This group was given 1 ml/kg of a 50% v/v solution of Carbon tetrachloride (Nice Chemicals Pvt. Ltd., Cochin) in olive oil intraperitoneally.

Group-III: This group was given Liv. 52 syrup (2 ml/kg) orally for twenty days followed by CCl₄ intraperitoneally as in Group-II. The Liv. 52 was administered by gavage method with animals fasted 3-4 hours prior and 1 hour after administration to ensure proper absorption. CCl₄ dose was given concomitantly with the last (20th day) dose of Liv. 52.

Group-IV: This group received the *S. nigrum* extract in the dose of 2ml/100g/day twice daily orally for the duration of 10 days followed by CCl₄ intraperitoneally as in Group-II on the last day.

Group-V: This group was given the *S. nigrum* extract (2ml/100g/day per orally twice daily) for the duration of 20 days followed by CCl₄ intraperitoneally as in Group-II on the last day.

Groups-VI: This group received the *S. nigrum* extract (2ml/100g/day per orally twice daily) for the duration of 30 days followed by CCl₄ intraperitoneally as in Group-II on the last day.

Animals of all the groups were fasted for 24 hours (during this duration water remained freely available) after which they were sacrificed under Ketamine (75 mg/kg) and Diazepam (10 mg/kg) anaesthesia given intraperitoneally⁴. Blood was collected from the anaesthetized animals from retro-orbital plexus. After blood collection the animals were sacrificed to obtain liver for histopathological examination.

Biological study parameters: The collected blood, after a standing time of half an hour, was centrifuged in Remi R-8 centrifuge at 2500 rpm for 10 min. The serum so obtained was used to estimate the intended biochemical study parameters viz. alanine transaminase (ALT) based on 2, 4-dinitrophenylhydrazine method⁵, alkaline phosphatase (ALP) based on Kind and King's method⁶ and Total bilirubin based on modified Jendrassik and Grof's method⁷.

TABLE 1: EFFECT OF LIV.52 (2 ML/KG/DAY, PO) AND SOLANUM NIGRUM (SN) (2 ML/100G/DAY, PO) FOR THE DURATION OF 10, 20 AND 30 DAYS, ON CARBON TETRACHLORIDE (CCl₄) INDUCED CHANGES IN VARIOUS BIOCHEMICAL PARAMETERS (n=6).

TREATMENT	ALANINE TRANSAMINASE (IU/l) (Mean ± SD)	ALKALINE PHOSPHATASE (IU/l) (Mean ± SD)	TOTAL BILIRUBIN (mg/dl) (Mean ± SD)
Normal Saline	25.87±5.30	23.73±11.26	0.25±0.11
Carbon Tetrachloride (1 ml/kg, ip)	423.08±22.02 [^]	237.84±35.83 [^]	1.88±0.21 [^]
Liv.52 + CCl ₄ on 20 th day	105.75±7.06 [*]	67.69±11.63 [*]	0.60±0.07 [*]
SN x 10 days + CCl ₄ on 10 th day	298.74±19.42 [*]	147.60±16.30 [*]	1.43±0.23 [*]
SN x 20 days + CCl ₄ on 20 th day	186.64±18.82 [*]	119.46±11.31 [*]	1.25±0.11 [*]
SN x 30 days + CCl ₄ on 30 th day	104.54±13.90 ^{*z}	72.10±14.31 ^{*z}	0.80±0.10 ^{*z}

[^]p<0.001 as compared to normal saline treated group. ^{*}p<0.05 as compared to CCl₄ treated group. ^zp>0.05 as compared to LIV.52 treated group.

Administration of aqueous extract of *S. nigrum* exhibited dose dependent limitation of ALT rise after CCl₄ administration.

A highly significant (p<0.001) rise in serum ALP levels was seen in CCl₄ treated group as compared to the normal saline treated group. The rise in serum ALP was significantly lower (p<0.001) in Liv. 52 treated group after CCl₄ administration as compared to the group

Histopathology: The liver was excised from the animals and washed with normal saline. A piece of about one cm was cut and fixed in 10% neutral formalin for 12-24 hours. It was then dehydrated and cleared with ethanol and xylene respectively followed by embedding in paraffin wax from which blocks were prepared. Sections of 5µm thickness were prepared from the blocks using a microtome⁸. These were processed in alcohol-xylene series and were stained with Harris haematoxylin and eosin stain⁹ and subjected to histopathological examination.

Statistical Analysis: Results were expressed as Mean ± Standard deviation (SD). Statistical differences between the groups were tested by one way analysis of variance (ANOVA) followed by Newman-Keuls Multiple Comparisons. P-values were estimated by referring to appropriate tables.

RESULTS: ALT level in normal saline treated group was 25.87±5.30 IU/l. It was found to be significantly increased (p<0.001) following administration of CCl₄ while pretreatment with known hepatoprotective preparation Liv. 52 significantly (p<0.001) limited the rise in ALT levels after CCl₄ administration (**table 1**).

which received only CCl₄. The effect of *S. nigrum* treatment on serum ALP levels was dose related and exhibited a trend similar to that seen in case of ALT.

As with ALT, the doses of 2ml/100g for 10, 20 and 30 days of *S. nigrum* produced significantly lesser (p<0.001) increments in serum ALP as compared to the CCl₄ treated group. In the doses of 2ml/100g for 30 days, effect of *S. nigrum* on serum ALT and ALP level was comparable to that of Liv. 52.

The administration of CCl_4 significantly increased ($p < 0.001$) the serum bilirubin as compared to normal saline treated group. The rise in serum bilirubin was significantly low ($p < 0.001$) in Liv. 52 treated group after CCl_4 administration as compared to only CCl_4 treated group. *S. nigrum* in the dose of 2ml/100g for 10 days produced less increment of serum bilirubin when compared to CCl_4 treated group ($p < 0.05$) while *S. nigrum* in the dose of 2ml/100g for 20 and 30 days ($p < 0.001$) showed significant limitation of serum bilirubin rise (Table 1).

Effect on Histology: Histology of liver of normal saline treated group displayed normal liver architecture. The hepatic cords and the sinusoids were well visible (Fig. 1).

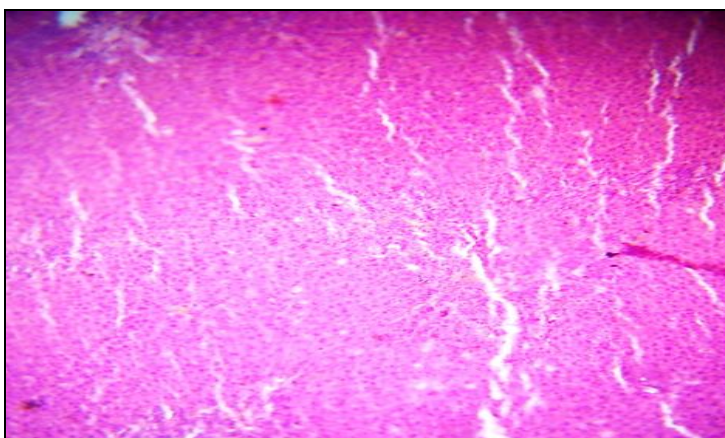


FIGURE 1: MICROSCOPIC FEATURES OF THE LIVER OF NORMAL SALINE TREATED GROUP (GR I) NORMAL HEPATIC LOBULE ARCHITECTURE IS SEEN. HEPATOCYTES AND THEIR NUCLEI ARE WELL VISIBLE

Classical centrilobular necrosis was seen in the CCl_4 treated group. The hepatocytes around the central vein were necrosed with no distinguishable nuclei (Fig. 2).

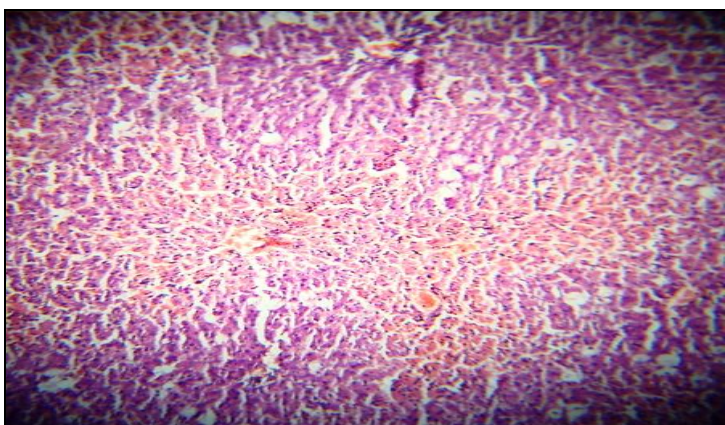


FIGURE 2: MICROSCOPIC FEATURES OF THE LIVER OF ANIMALS TREATED WITH CCl_4 (GR II) EXTENSIVE CENTRIZONAL NECROSIS IS SEEN. NO HEPATOCYTES WITH NUCLEI ARE DISCERNIBLE

Liv. 52 treated group revealed very mild signs of liver injury. Only difference from the normal saline treated group was the presence of inflammatory cells and constricted sinusoids indicating apparent hepatocyte swelling (Fig. 3).

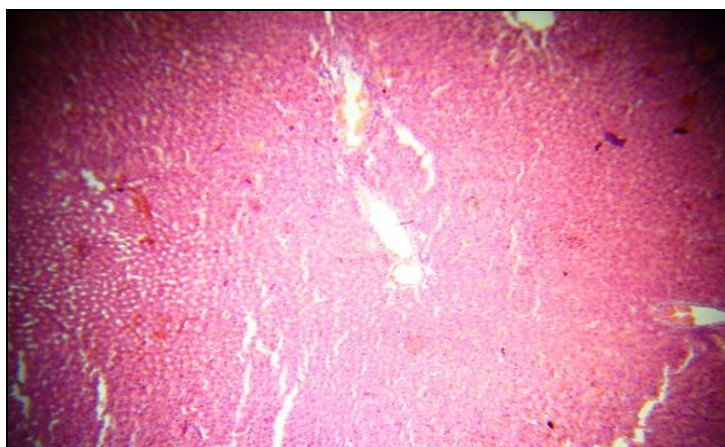


FIGURE 3: MICROSCOPIC FEATURES OF THE LIVER OF ANIMALS TREATED WITH LIV.52. (GR III) MILD HEPATOCYTE SWELLING IS PRESENT AS INDICATED BY CONSTRICTED SINUSOIDS. INFLAMMATORY CELLS ARE SEEN AROUND CENTRAL VEINS. NECROSIS IS ABSENT

The group treated with aqueous extracts of *S. nigrum* in a dose of 2 ml/100g for 10 days showed feathery degeneration in the centrilobular area which was the predominant histological feature of this group (Fig. 4).

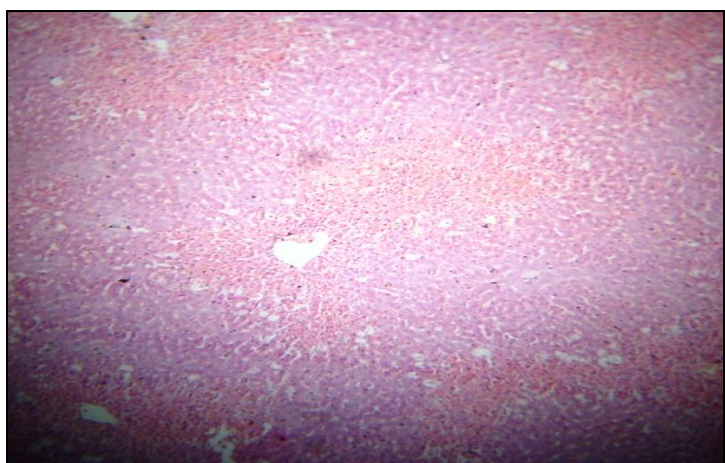


FIGURE 4: MICROSCOPIC FEATURES OF THE LIVER OF ANIMALS TREATED WITH *SOLANUM NIGRUM* AQUEOUS EXTRACT 2ML/100G/D FOR 10 DAYS (GR IV). FEATHERY DEGENERATION OF HEPATOCYTES IS SEEN AROUND THE CENTRAL VEIN

The primary feature in the groups treated with 2 ml/100g of *S. nigrum* for 20 days was the presence of fatty changes in the centrilobular area. Necrosis was mild (Fig. 5).

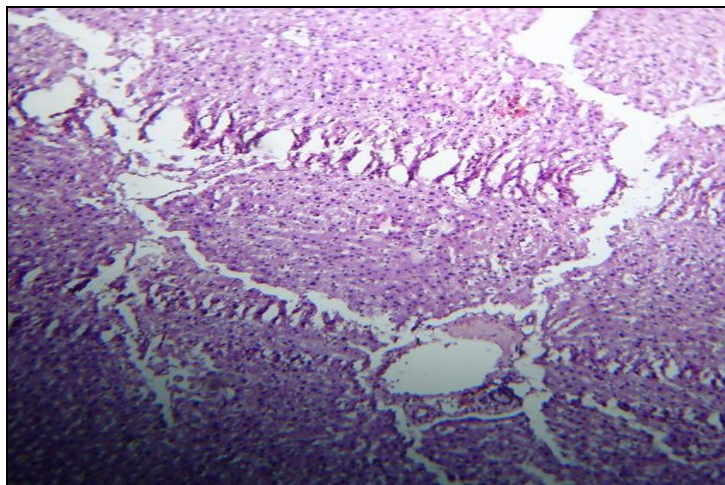


FIGURE 5: MICROSCOPIC FEATURES OF THE LIVER OF ANIMALS TREATED WITH SOLANUM NIGRUM AQUEOUS EXTRACT 2ML/100G/D FOR 20 DAYS (GR V). CENTRIZONAL AREA IS CONSPICUOUS BY THE PRESENCE OF HEPATOCYTES SHOWING FATTY CHANGES. MILD NECROSIS SEEN

Almost normal hepatic lobule architecture was seen in the group treated with 2 ml/100g of *S. nigrum* for 30 days. Similar to the Liv.52 treated group, this group showed the presence of constricted sinusoids, pointing towards mild hepatocyte swelling. No necrosis or inflammation was seen. Congested central vein was seen (**Fig. 6**).

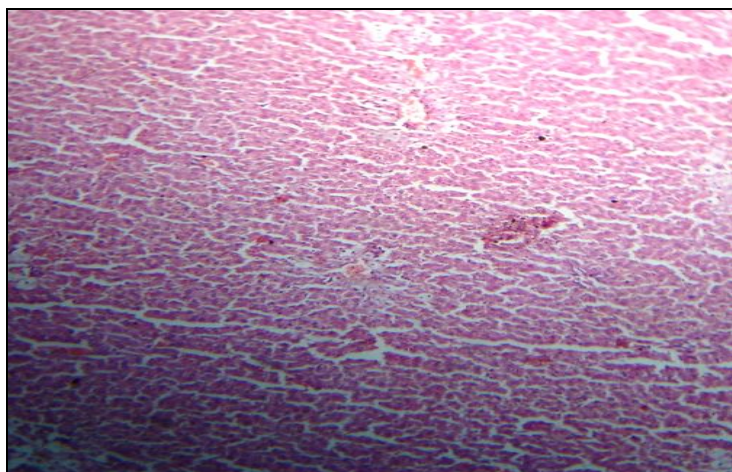


FIGURE 6: MICROSCOPIC FEATURES OF THE LIVER OF ANIMALS TREATED WITH SOLANUM NIGRUM AQUEOUS EXTRACT 2ML/100G/D FOR 30 DAYS (GR VI)). CONSTRICTED SINUSOIDS ARE SEEN POINTING TO MILD HEPATOCYTE SWELLING. NECROSIS IS ABSENT

DISCUSSION: Liver damage induced by CCl_4 is a commonly used model for the screening of hepatoprotective drugs¹⁰. CCl_4 is metabolically activated by the cytochrome P-450 dependent mixed oxidase in the endoplasmic reticulum to form trichloromethyl free radical (CCl_3) which combines with cellular lipids and proteins in the presence of oxygen to induce lipid peroxidation^{11, 12}. These result in changes in structures of the endoplasmic reticulum and

membranes of other organelles, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose 6 phosphatase activation, leading to liver injury and elevated levels of transaminases, alkaline phosphatase, bilirubin etc.^{13, 14, 15}.

Serum alanine transaminase, and alkaline phosphatase were found to be significantly elevated after CCl_4 administration (Table 1) though the rise in bilirubin level was not to the same extent as ALP and ALT^{16, 17}. This could be explained by the fact that bilirubin reaches peak serum level in the second hour after CCl_4 administration and probably declines afterwards¹⁸.

Blood collection in the present study was 24 hours after CCl_4 administration and thus, the serum bilirubin levels would have been on the decline (Table 1). Further, histopathology showed severe centrilobular necrosis, hepatocyte necrosis, portal inflammation, inflammatory cell infiltration, and macro and micro-vesicular steatosis (Fig. 2). The preferential affection of zone 3 may be attributed to the enzyme specificity and metabolic heterogeneity of hepatocytes as suggested by Gumucio¹⁹.

It was observed, that Liv. 52 significantly suppressed the rise of ALT and ALP after CCl_4 challenge. It also normalized the bilirubin levels (Table 1). This biochemical protection was also reflected in the histology which showed only mild hepatocellular swelling with the presence of some inflammatory cells (Fig. 3).

S. nigrum exhibited dose dependent hepatoprotection, both biochemically and histologically (Table 1, Fig. 4, 5 & 6). It is interesting to note that the *S. nigrum* extract (2ml/100g) for 30 days provided better results with ALP as compared to Liv. 52. The same dose for same duration gave good protection against an increase in ALT following CCl_4 administration, though level of protection was slightly less as compared to Liv. 52. In all, *S. nigrum* treated groups and Liv. 52 treated group, the bilirubin levels were in normal range.

Since formation of free radicals by cytochrome P450 after metabolism of CCl_4 ²⁰ has been implicated in lipid peroxidation mediated hepatocyte injury, the effect on lipid peroxidation and a stimulatory effect on hepatic regeneration as well^{21, 22}.

The pharmacological constituents separated out from *Solanum nigrum* are reported to be steroidal glycoalkaloids (solamargine, solasonine, solanigrine), steroidal genin (titogenin), saponins and vitamins (riboflavin, nicotinic acid, ascorbic acid and carotene)²³.

The possible mechanism for hepatoprotective activity of Liv. 52 has been ascribed to its inhibitory effect on the microsomal enzymes so that generation of free radicals is limited. Liv. 52 is also claimed to act as a free radical scavenger thereby preventing lipid peroxidation. Another suggested mechanism for hepatoprotective activity of Liv. 52 is its anti-oxidant property, on account of which it may exert an inhibitory effect on lipid peroxidation and a stimulatory effect on hepatic regeneration as well.

The possible mechanism for hepatoprotective activity of aqueous extract of *S. nigrum* is not clearly known. It is assumed that the effect of *S. nigrum* extract on liver protection may be due to several reasons. It may be related to glutathione-mediated detoxification. *S. nigrum* could have enhanced GSH status in cells and thereby afforded protection to hepatic cells from toxic damages.

The effects of silymarin, a proven hepatoprotective agent, are mainly attributable to its antioxidant and free radical scavenging properties²⁴. Like silymarin, *S. nigrum* might also act as an antioxidant and a free radical scavenger.

Some still unexplained mechanisms of *S. nigrum* may be assumed to be involved in protecting liver from carbon tetrachloride induced toxicity. The hepatoprotective activity of *S. nigrum* aqueous extract was comparable in the dose of 2 ml/100g/day for 30 days to that with Liv. 52 syrup (2 ml/kg) for 10 days. This fact does not undermine the efficacy of the *S. nigrum* aqueous extract because; the Liv. 52 is a hydroalcoholic extract. Thereby, a hydroalcoholic extract of *S. nigrum* can be expected to deliver these results at much smaller doses and for less duration. Further the pharmacokinetic studies of *S. nigrum* are largely unknown. An elaborate investigation to explore the pharmacokinetic profile may lead to better efficacy and potency of *S. nigrum* at smaller doses.

CONCLUSION: It can be concluded from the present study, that *S. nigrum* aqueous extract is a potent hepatoprotective agent. Further studies of extracts of *S. nigrum* should be conducted for the isolation and structure determination of the active hepatoprotective principle(s). Though in this study, the aqueous extract of *S. nigrum* was used, other forms like hydroalcoholic extract or extraction of some specific components which are involved in hepatoprotective action can also be used in future studies.

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