

ORIGINAL CONTRIBUTION

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Solanum nigrum confers protection against CCl₄-induced experimental hepatotoxicity by increasing hepatic protein synthesis and regulation of energy metabolism

Rajesh Krithika* and Ramtej Jayaram Verma

Abstract

Background: Hepatotoxicity induced by carbon tetrachloride (CCl₄) is used as an experimental model to screen phytochemicals with liver protecting activity. *Solanum nigrum* (SN) is a shrub which is widely distributed throughout India. Traditionally the plant has been used for curing various ailments related to gastroenterology and hepatology. The present study was performed to evaluate the hepatoprotective property of *S. nigrum* extract standardized for its flavonoid content against CCl₄-induced hepatotoxicity.

Methods: The liver protecting property of SN was evaluated by means of various biochemical parameters and histopathologically. *S. nigrum* was administered to Swiss strain female albino mice with either 100/ 200/ 300 mg/kg body weight/day for 30 days along with CCl₄ which is a well established model to induce hepatotoxicity.

Results: Administration of CCl₄ for 30 days caused a significant increase in liver marker enzymes and a decrease in hepatic DNA, RNA and protein levels which was effectively mitigated by treatment with the plant extract in a dose dependent manner. Similarly co-treatment of the extract along with the hepatotoxin improved hepatic energy status by increasing the activities of succinate dehydrogenase (SDH) and adenosine triphosphatase (ATPase). Histopathological findings indicated severe vacuolization and necrotic changes after CCl₄ treatment which was mitigated by the co-administration of SN extract in a dose-dependent manner.

Conclusions: Carbon tetrachloride is known to exert hepatotoxicity by forming adducts with tissue macromolecules through covalent interactions. *S. nigrum* extract was found to effectively mitigate CCl₄-induced changes in hepatic macromolecular content and energy status of liver tissue. The present study has identified the standardized *S. nigrum* as an effective hepatoprotective agent probably due to its role in improving the protein and energy levels in the hepatic tissue.

Keywords: *S. nigrum*, CCl₄, Hepatoprotection, Protein synthesis, Energy metabolism, Histopathology

Background

Carbon tetrachloride (CCl₄) once used as an industrial solvent, is presently used as an experimental agent to induce hepatotoxicity. This agent has been used successfully studies both in vitro and in vivo in many species to produce liver injury. It is well established that CCl₄ is metabolically activated in the liver to produce intermediates

which are then dealt by the hepatic mixed function oxidases and some related enzyme systems. Metabolism of CCl₄ by mixed function cytochrome P450 oxygenase system of the endoplasmic reticulum and especially by CYP2E1 generates trichloromethyl free radical (CCl₃·) which has the capacity to interact with critical target biomolecules like DNA, lipids and proteins via covalent bond formation. The reductive metabolism of CCl₄ to the CCl₃· free radical thus results in the production of covalently bound adducts and have been reported to be responsible for CCl₄-induced hepatic necrosis [1]. It is widely accepted

* Correspondence: krithiraj2002@yahoo.co.in; krithiraj2002@gmail.com
Department of Zoology, Biomedical Technology and Human Genetics,
University School of Sciences, Gujarat University, Navrangpura, Ahmedabad
380009, India

that CCl_4 -induced experimental hepatotoxicity greatly mimics viral hepatitis [2]. Moreover CCl_4 can also induce hepatotoxicity via lipid peroxidation mechanism through trichloromethyl peroxy radical ($\text{CCl}_3\text{OO}\cdot$) which is formed when $\text{CCl}_3\cdot$ reacts with O_2 [3].

Consumption of herbal medications against diseases is on a high and a very sharp rise in its usage has been reported by patients suffering from liver diseases [4]. Medicinal plants can act as supreme agents against any form of liver ailments. Thus treating liver diseases with plant derived compounds seems to be very lucrative option especially considering the fact that it does not require any laborious or vigorous process for production or development and is also cost effective and hence can be considered as supreme liver protecting agents. Hence treatment of liver diseases with plant-derived compounds which are highly accessible and do not require laborious pharmaceutical synthesis seems highly attractive.

S. nigrum has been commonly used as a traditional medicine for treating various diseases. It has been used to treat inflammation, oedema and other related disorders since time immemorial. It is commonly known as 'Black nightshade' and has been extensively used in traditional medicine in India and other parts of world to cure liver disorders, chronic skin ailments (psoriasis and ringworm), inflammatory conditions, painful periods, fevers, diarrhea, eye diseases, hydrophobia and as a folk medicine for treatment of peptic ulcer [5]. In traditional medicine, the plant has also been used as an anti-diarrhoeal, in eye diseases and hydrophobia [6]. Chinese medicine employs the juice of the leaves to alleviate pain in inflammation of the kidney and bladder and internally for cardialgia [5].

Many previous studies have been carried out to determine the hepatoprotective effect of different extracts of *S. nigrum* against different toxicants. The anti-fibrotic role of *S. nigrum* against thioacetamide-induced toxicity has been reported earlier [7]. Raju et al. 2003, have reported that the dried fruits of *S. nigrum* possess anti-hepatotoxic potential when administered for 7 days on a pre-treatment basis against a single oral dose of CCl_4 [8]. Similarly *S. nigrum* when administered for 10 days on a co-treatment basis was found to ameliorate the drastic increase in hepatic markers and was able to reduce the histopathological anomalies and restore back normalcy in haematological parameter induced by CCl_4 in rats [9]. Mir et al. 2010, have evaluated the hepatoprotective property of *S. nigrum* against CCl_4 -induced acute toxicity in rats [10]. Hepatotoxicity was induced by administering four oral dose of CCl_4 within 24 h interval period and subsequent administration of 4 oral doses of *S. nigrum* extract (500 mg/kg body weight) resulted in marked reduction in liver enzymes and improved histopathological changes especially the fatty changes and prevented liver enlargement.

The protective effect of water extract of *S. nigrum* against CCl_4 -induced chronic hepatotoxicity in rats has been evaluated [11]. *S. nigrum* was found to reduce CCl_4 -induced lipid peroxidation by modulating the antioxidative defence mechanism. Elshater et al. 2013, have reported that rats treated with *S. nigrum* extract for 30 days after challenge with CCl_4 (1 mg/kg body weight dose thrice per week for 2 weeks) resulted in significant reduction in liver marker enzymes, lipid peroxidation and an elevation in enzymatic and non-enzymatic antioxidative defence system [12].

Pre-treatment with *S. nigrum* extract for 7 days of was shown to reduce the levels of liver marker enzymes, decrease lipid peroxidation and improve histopathological features in rats administered with a single ip dose of CCl_4 [13]. The hepatoprotective potential of aqueous extract of *S. nigrum* on pre-treatment basis for 10–30 days against toxicity induced by a single dose of CCl_4 has been studied which indicated that treatment *S. nigrum* extract with CCl_4 caused marked elevation in the levels of ALT, ALP and bilirubin along with distortion in histopathological structure [14]. *S. nigrum* is thus known to exert its effect by curtailing lipid peroxidation due to its antioxidative property Sivgami et al. 2012 [15].

The present study was carried out to evaluate the mitigatory effect of standardized extract of *S. nigrum* against CCl_4 -induced changes in hepatic biomolecules via adduct formation and deterioration of hepatic energy status. *S. nigrum* was administered on a co-treatment basis in the present study against CCl_4 -induced sub-chronic hepatic toxicity to address the shortcomings of most of the previous studies performed to evaluate the protective action of the plant extract in which hepatotoxicity was induced by a single dose of CCl_4 after pre-treatment with the extract.

Methods

Animals

The experiments were performed in inbred Swiss strain female albino mice (*Mus musculus*) weighing between 32 and 35 g (6–8 weeks old) obtained from Zydus Research Centre (Ahmedabad, India). All animals were maintained under controlled conditions of temperature ($25 \pm 2^\circ\text{C}$) and relative humidity (50–55%) and under a 12 h light / dark cycle in the Animal House of Zoology Department, Gujarat University (Ahmedabad, India). Animals were fed certified rodent diet supplied by Amrut Feeds, Pranav Agro Industries Limited (Pune, India) and water ad libitum. Formal approval was taken from "The Committee for the Purpose of Control and Supervision of Experiment on Animals" (Reg – 167/1999/ CPCSEA), New Delhi, India for performing the experiments and the animals were cared strictly abiding to the guidelines of imposed by Prevention of Cruelty to

Animals Act, 1960 (59 of 1960; Government of India, New Delhi).

Chemicals

All chemicals used in the entire study were procured from standard agency. HPLC grade CCl_4 and other solvents were obtained from Merck Specialities Pvt. Ltd. (Mumbai, India) and olive oil from Figaro (Madrid, Spain).

Experimental protocol

Plant material

The aerial parts of *S. nigrum* (SN) bearing ripe fruits were obtained from Botanical Garden of Gujarat University (Ahmedabad, India). The identity of the plant materials was confirmed by Dr. A. K. S. Rawat, Scientist and Head, Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute (NBRI), Lucknow, India. The aerial parts were shade dried, powdered and soaked with ethanol: water (3×1000 mL, 50:50, v/v) at room temperature. The solvent was evaporated under reduced pressure in a rotary evaporator at 40°C . The *S. nigrum* extract was standardized for its flavonoid content before its administration to experimental animals. The total flavonoid content (%) of the SN extract calculated in terms of quercetin equivalence by plotting a calibration curve was measured at 510 nm in triplicates using quercetin standard [16].

Animal groupings

The mice were divided into seven groups consisting of ten animals each. Group 1 mice were marked as untreated control and were given free access to feed and drinking water. Animals of group 2 (vehicle control) received 0.2 mL of olive oil which was used as the vehicle to dissolve CCl_4 . Animals of groups 3 was given *S. nigrum* (SN) extract standardized on flavonoid content (300 mg /kg body weight, p.o.) which served as plant control group. Group 4 animals were administered with CCl_4 (826 mg/0.2 mL olive oil/kg body weight/day, p. o.). Groups 5–7 were orally treated with CCl_4 along with 100, 200 and 300 mg/kg body weight/day of standardized SN extract.

Treatment was continued for 30 days in all groups and mice were also observed for any behavioural or clinical changes during the course of treatment. The body weights of mice were individually recorded after completion of treatment and mean weights were calculated. Blood was collected by cardiac puncture on 31st day and centrifuged at $1000 \times g$ for 10 min to obtain serum. Once the animals were sacrificed by cervical dislocation the liver was dissected out quickly, blotted free of blood and used for histopathological examinations and biochemical analysis.

Histopathological examination

Tissues for histopathological examination were preserved in 10% neutral buffered formalin immediately after autopsy. Standard technique for hematoxylin and eosin (H & E) staining was followed by dehydrating the tissues in ascending grades of alcohol, clearing them in xylene and embedding in paraffin wax. $5 \mu\text{m}$ thick sections were cut on a rotary microtome and stained in H & E, treated with alcohol, cleared in xylene and mounted in DPX and examined under a light microscope.

Assessment of liver function

The alanine transaminase (ALT) activity and aspartate transaminase (AST) in liver homogenate and serum were determined [17]. The alkaline phosphatase (ALP) activity in both liver and serum was estimated [18]. Alkaline phosphatase at optimum pH 10.5 catalyzes the hydrolysis of p-nitrophenyl phosphate (disodium salt) to p-nitrophenol and inorganic phosphate. The liberated p-nitrophenol combines with sodium hydroxide to form a yellow coloured complex which was measured at 410 nm. Similarly the activity of acid phosphatase (ACP) was assayed in liver and serum by the method as described in Sigma Technical Bulletin [19]. Acid phosphatase at optimum pH 4.8 catalyzes the hydrolysis of p-nitrophenyl phosphate (disodium salt) to p-nitrophenol and inorganic phosphate. The liberated p-nitrophenol combines with sodium hydroxide to form a yellow coloured complex which was measured at 420 nm.

Serum lactate dehydrogenase (LDH) activity was determined by the method of King (1965) [20]. The method is based on the ability of LDH to convert lactate to pyruvate with the help of coenzyme nicotinamide adenine dinucleotide (NAD^+). The pyruvate formed is made to react with 2, 4-dinitrophenyl hydrazine in hydrochloric acid. The hydrazone formed in alkaline medium was read at 540 nm. The γ -glutamyl transpeptidase (γ -GT) activity in serum was also estimated following the method of Orłowski and Meister (1965) [21]. This enzyme catalyzes transfer of gamma glutamyl groups from gamma glutamyl peptides to suitable acceptor. The enzymatic reaction in the presence of substrate γ -glutamyl-p-nitroaniline results in the formation of p-nitroaniline whose release was monitored by noting increase in absorbance at 410 nm.

Estimation of nucleic acids and proteins content

A known weight of liver was homogenized in 5 mL of cold 5% TCA and homogenate was kept at $0-4^\circ\text{C}$ for 30 min and subsequently processed in alcohol: ether (1:3, v/v) and centrifuged to obtain lipid free pellet which was dissolved in of 0.1 N KOH and incubated at 37°C for 16–18 h to will 6 N HCl and 10% TCA were added and centrifuged. The supernatant was used for RNA

estimation by the method of Schneider (1945) using orcinol reagent to give a greenish colour, which was read at 670 nm [22]. To the pellet 5% TCA was added and heated at 90 °C for 15 min. The supernatant was then separated by centrifugation (10 min at 1000 x g) and used for DNA estimation by the method of Giles and Meyer (1965) [23]. The DNA in the supernatant reacts with diphenylamine to give blue coloured complex whose optical density was read at 620 nm. The protein content of the liver was estimated by the method of Lowry et al. (1951) [24].

Estimation of hepatic succinate dehydrogenase (SDH) and adenosine triphosphatase (ATPase) activity

The liver succinate dehydrogenase (SDH) activity was measured by the method of Beatty et al. 1966, using 2-(4-iodophenyl)-3(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (INT) as an electron acceptor [25]. The electrons released by the enzyme from the substrate are taken up by INT, which was reduced to a red coloured formazon. This was extracted in ethyl acetate and measured at 420 nm. The hepatic adenosine triphosphatase (ATPase) activity was assayed by using the method of Quinn and White (1968) [26]. ATPase causes hydrolysis of adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic phosphate (i.p.). The liberated inorganic phosphorus was estimated by the method of Fiske and Subbarow (1925) [27]. The optical density was read at 660 nm.

Statistical analysis

The results were analyzed by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparison post-hoc tests using the 19th version of SPSS software. The level of significance was accepted with * $p < 0.001$.

Results

Total flavonoid content

The total flavonoid content (%) of the standardized S. nigrum extract was determined to be 1.75 ± 0.17

Changes in body weight Table 1 represents the result of plant extracts treatment on CCl₄-induced changes in the body weight of mice. No significant difference in body weight was observed between different control groups (Groups 1–3). However, CCl₄ treatment for 30 days (Group 4), as compared to vehicle control (group 2), caused a significant ($p < 0.001$) reduction in body weight. Oral administration of CCl₄ along-with hydroalcoholic extracts of SN (Groups 5–7) caused significant increase ($p < 0.001$) in body weight, as compared to CCl₄ alone treated animals (Group 4) in a dose-dependent

Table 1 Effect of *S. nigrum* on CCl₄-induced changes in body weight (gm) of mice

Experimental groups	Days of treatment	
	0	30
Control		
1. Untreated	32.87 ± 1.65	34.99 ± 1.67
2. Olive oil	32.04 ± 0.15	34.74 ± 0.94
3. <i>S. nigrum</i> (SN)	32.49 ± 0.94	34.93 ± 1.32
Carbon Tetrachloride (CCl ₄)-Treated		
4. CCl ₄	32.52 ± 1.23 ^{a*}	27.43 ± 1.59 ^{a*}
CCl ₄ + <i>Solanum Nigrum</i> (SN) Extract-Treated		
5. CCl ₄ + SN100	33.03 ± 1.32 ^{b*}	30.11 ± 0.99 ^{b*}
6. CCl ₄ + SN200	32.66 ± 1.12 ^{b*}	31.13 ± 0.45 ^{b*}
7. CCl ₄ + SN300	33.04 ± 0.98 ^{b*}	33.82 ± 0.53 ^{b*}

Results are expressed as mean ± SEM; n = 10

No significant difference was noted between groups 1–3

^aas compared between vehicle control (group 2) and toxin-treated (group 4),

^bas compared between toxin-treated (group 4) and toxin + antidote-treated (groups 5–7)

Level of significance * $p < 0.001$

manner. The 300 mg/kg body weight dose was the most effective in increasing the body weight of mice.

Histopathology Liver sections (H & E stained) of vehicle control mice (Fig. 1a) showed normal structure of central vein with radially arranged normal hepatocytes. No apparent histopathological changes were observed in the liver of plant extracts alone treated mice (Fig. 1b). Oral administration of HD of CCl₄ for 30 days caused hepatocellular necrosis and cytoplasmic vacuolization (Fig. 1c). On the other hand oral administration of SN alongwith CCl₄ caused significant, dose-dependent protection against the changes induced by the hepatotoxin (Fig. 1 d-e). Dilation of central vein and distortion of hepatocytes became less apparent in liver of SN300 plus CCl₄ (Fig. 1f) administered mice. Fatty degenerative changes were also less pronounced in SN300 and CCl₄-treated mice (Figs. 1f).

Liver biochemical analysis Table 2 represents the results of CCl₄-induced changes in activities of some liver enzymes of mice with and without plant extract treatment. Results revealed no significant changes in the activities of ALT, AST, ALP and ACP between different control groups (Groups 1–3). However, CCl₄ treatment for 30 days (Group 4), as compared to vehicle control (group 2), caused significant ($p < 0.001$) increase in all these parameters. Oral administration of three different doses of SN alongwith CCl₄ (Groups 5–7) significantly ($p < 0.001$) ameliorated the CCl₄-induced changes. The effect was dose-dependent and maximum at the dose regimen of 300 mg/kg body weight.

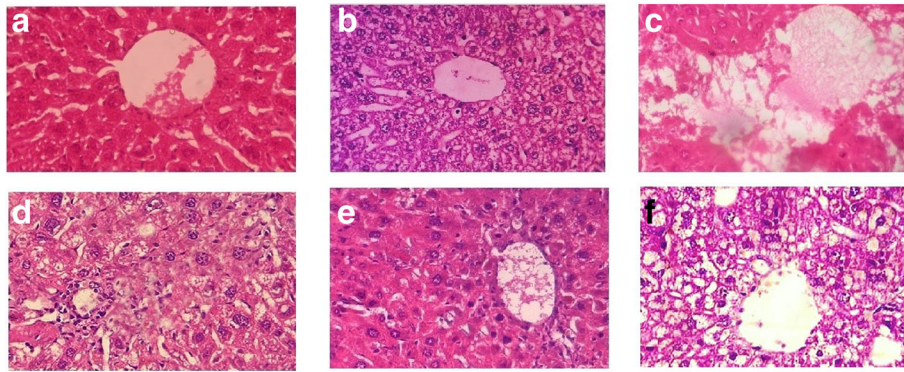


Fig. 1 Light micrographs (H & E stained) of liver (a) vehicle control mice showing normal architecture with radially arranged central vein (200 x) (b) *S. nigrum* control mice showing normal structure and absence of vacuolization and fatty degenerative changes (200x) (c) CCl₄-treated indicating extensive cytoplasmic vacuolization and fatty infiltration (200x) (d) *S. nigrum* 100 mg/kg bwt plus CCl₄-treated mice showing moderate necrotic changes and fatty degenerative changes and ballooning of hepatocytes (e) *S. nigrum* 200 mg/kg bwt plus CCl₄-treated mice showing mild centrilobular necrosis degenerative changes (200x) (f) *S. nigrum* 300 mg/kg bwt plus CCl₄-treated mice showing normal arrangement of hepatocytes and almost similar to vehicle control mice (400 x)

Serum biochemical parameters Table 3 shows the results of plant extract treatment on CCl₄-induced changes in the activities of marker enzymes in serum of mice. No significant difference in the activities of ALT, AST, ALP, ACP, LDH and γ-GT were observed among all control group animals (Groups 1–3). It was observed that activities of all serum marker enzymes were significantly ($p < 0.001$) increased after oral administration of HD CCl₄ (Group 4) as compared to vehicle control (Group 2) which was effectively reversed in a dose-dependent manner when SN extract was given along with the toxin (Groups 5–7).

Hepatic nucleic acid, protein content and energy status Table 4 presents the results of mitigatory effects

of plant extracts on biochemical changes induced by CCl₄ in liver of mice. No significant change was observed in DNA, RNA and protein contents in different control groups (Groups 1–3). Conversely, oral administration of HD of CCl₄ (Group 4) caused a significant ($p < 0.001$) decrease in these parameters. Results revealed that co-treatment of SN along with CCl₄ (Groups 5–7), significantly ($p < 0.001$) ameliorated the toxin-induced changes in DNA, RNA and protein content in a dose-dependent manner.

Table 5 depicts the results of CCl₄-induced changes in energy metabolism. No significant changes were observed in the activities of SDH and ATPase in liver of control mice (Groups 1–3). However, a significant reduction was noted in the activities of SDH and ATPase

Table 2 Effect of *S. nigrum* on CCl₄ -induced changes in enzymatic parameters in mice liver

Experimental groups	ALT	AST	ALP	ACP
Control				
1. Untreated	1.69 ± 0.16	1.94 ± 0.39	0.27 ± 0.08	1.28 ± 0.72
2. Olive oil	1.60 ± 0.18	1.95 ± 0.27	0.28 ± 0.06	1.27 ± 0.54
3. <i>S. nigrum</i> (SN)	1.56 ± 0.13	1.96 ± 0.22	0.30 ± 0.09	1.28 ± 0.11
Carbon Tetrachloride (CCl ₄) -Treated				
4. CCl ₄	3.89 ± 0.23 ^{a*}	4.61 ± 0.81 ^{a*}	0.59 ± 0.08 ^{a*}	2.89 ± 0.44 ^{a*}
(V) CCl ₄ + <i>Solanum Nigrum</i> (SN) Extract-Treated				
5. CCl ₄ + SN100	2.78 ± 0.32 ^{b*}	3.80 ± 0.98 ^{b*}	0.36 ± 0.07 ^{b*}	1.94 ± 0.33 ^{b*}
6. CCl ₄ + SN200	2.51 ± 0.32 ^{b*}	3.59 ± 0.32 ^{b*}	0.35 ± 0.09 ^{b*}	1.88 ± 0.12 ^{b*}
7. CCl ₄ + SN300	2.09 ± 0.66 ^{b*}	2.73 ± 0.21 ^{b*}	0.33 ± 0.11 ^{b*}	1.58 ± 0.43 ^{b*}

Results are expressed as mean ± SEM; n = 10

No significant difference was noted between groups 1–3

^aas compared between vehicle control (group 2) and toxin-treated (group 4),

^bas compared between toxin-treated (group 4) and toxin + antidote-treated (groups 5–7)

Level of significance * $p < 0.001$

Units: ALT - mU/mg protein/30 min; AST - mU/mg protein/60 min; ALP - μmoles p-nitrophenol released/mg protein/30 min; ACP - μmoles p-nitrophenol released/mg protein/30 min

Table 3 Effect of *S. nigrum* on CCl₄ -induced biochemical changes in serum of mice

Experimental groups	ALT	AST	ALP	ACP	LDH	γ-GT
Control						
1. Control	12.67 ± 0.35	39.67 ± 0.78	0.44 ± 0.02	0.21 ± 0.07	0.86 ± 0.09	0.78 ± 0.61
2. Olive oil	12.59 ± 0.43	39.72 ± 0.13	0.43 ± 0.08	0.20 ± 0.05	0.85 ± 0.01	0.78 ± 0.18
3. <i>S. nigrum</i>	12.28 ± 0.41	39.94 ± 0.16	0.44 ± 0.15	0.21 ± 0.07	0.86 ± 0.45	0.79 ± 0.13
Carbon Tetrachloride Treated (CCl ₄) -Treated						
4. CCl ₄	48.78 ± 1.08 ^{a*}	96.58 ± 1.72 ^{a*}	0.79 ± 0.12 ^{a*}	0.36 ± 0.03 ^{a*}	1.98 ± 0.32 ^{a*}	1.57 ± 0.21 ^{a*}
CCl ₄ + <i>Solanum Nigrum</i> (SN) Extract -Treated						
5. CCl ₄ + SN100	23.15 ± 0.04 ^{b*}	63.25 ± 0.98 ^{b*}	0.62 ± 0.05 ^{b*}	0.29 ± 0.06 ^{b*}	1.64 ± 0.11 ^{b*}	1.43 ± 0.56 ^{b*}
6. CCl ₄ + SN200	17.62 ± 0.76 ^{b*}	53.14 ± 1.09 ^{b*}	0.59 ± 0.07 ^{b*}	0.27 ± 0.07 ^{b*}	1.58 ± 0.56 ^{b*}	1.32 ± 0.65 ^{b*}
7. CCl ₄ + SN300	14.63 ± 0.66 ^{b*}	48.40 ± 1.11 ^{b*}	0.53 ± 0.04 ^{b*}	0.24 ± 0.07 ^{b*}	1.42 ± 0.98 ^{b*}	1.03 ± 0.32 ^{b*}

Results are expressed as mean ± SEM; n = 10

No significant difference was noted between groups 1–3

^aas compared between vehicle control (group 2) and toxin-treated (group 4),

^bas compared between toxin-treated (group 4) and toxin + antidote-treated (groups 5–7)

Level of significance *p < 0.001

Units: ALT - mU/mL; AST - mU/mL; ALP - μmoles p-nitrophenol released/mg protein/30 min; ACP - μ moles p-nitrophenol released/mg protein/30 min; LDH - μmoles pyruvate liberated/mg protein/min; γ-GT - μmoles p-nitroaniline liberated/mg protein/min

in CCl₄-treated animals (Group 4). The percent decrease in the SDH activity was more prominent as compared to ATPase. Results revealed a significant amelioration in the activities of SDH and ATPase when SN was concurrently administered with CCl₄ (Groups 5–7). The effects were dose-dependent.

Discussion

Oral administration of CCl₄ for 30 days caused a significant reduction in the body weight of mice (Table 1). Mice administered with CCl₄ were observed to be dull with reduced feed consumption which probably

contributed to the decrease in the body weight observed. A similar loss in body weight, dullness and anorexic behaviour in rats treated with CCl₄ has been reported by Abuelgasim et al. (2008) [28]. Plant extracts alone treated mice (group 3) did not show any significant difference in body weight as compared to vehicle control (group 2). Results indicated a significant and dose-dependent increase in body weight of plant extract plus CCl₄-treated mice. Lin et al. 2008, have reported a significant decrease in the body weight of rats administered with 0.5 mL of 20% (v/v) solution CCl₄ in corn oil, which was significantly mitigated by treatment with *S. nigrum* extract [11]. Hsieh

Table 4 Effect of *S. nigrum* on CCl₄ -induced biochemical changes in liver of mice

Experimental groups	DNA	RNA	Protein
Control			
1. Untreated	483.09 ± 5.68	326.15 ± 2.25	22.08 ± 1.23
2. Olive oil	483.61 ± 6.32	324.82 ± 1.67	22.14 ± 1.43
3. <i>S. nigrum</i> (SN)	482.91 ± 0.99	325.75 ± 4.45	22.89 ± 0.54
Car Carbon Tetrachloride (CCl ₄) -Treated			
4. CCl ₄	342.90 ± 2.65 ^{a*}	251.69 ± 2.78 ^{a*}	13.67 ± 0.87 ^{a*}
(V) C CCl ₄ + <i>Solanum Nigrum</i> Extract (SN) -Treated			
5. CCl ₄ + SN100	369.51 ± 1.87 ^{b*}	265.19 ± 0.95 ^{b*}	18.19 ± 0.55 ^{b*}
6. CCl ₄ + SN200	400.14 ± 1.21 ^{b*}	280.14 ± 1.09 ^{b*}	19.89 ± 0.98 ^{b*}
7. CCl ₄ + SN300	419.72 ± 0.76 ^{b*}	292.04 ± 1.21 ^{b*}	21.13 ± 0.88 ^{b*}

Results are expressed as mean ± SEM; n = 10

No significant difference was noted between groups 1–3

^aas compared between vehicle control (group 2) and toxin-treated (group 4),

^bas compared between toxin-treated (group 4) and toxin + antidote-treated (groups 5–7)

Level of significance *p < 0.001

Units: DNA - μmoles/100 mg tissue weight; RNA - μmoles/100 mg tissue weight; Protein - mg/100 mg tissue weight

Table 5 Effect of *S. nigrum* on CCl₄ -induced changes in SDH and ATPase activities

Experimental groups	SDH	ATPase
Control		
1. Untreated	61.12 ± 2.12	0.35 ± 0.12
2. Olive oil	61.89 ± 1.12	0.35 ± 0.09
3. <i>S. nigrum</i> (SN)	61.01 ± 0.44	0.34 ± 0.11
Carbon Tetrachloride (CCl ₄) -Treated		
4. CCl ₄	33.23 ± 1.24 ^{a*}	0.17 ± 0.02 ^{a*}
(V) CCl ₄ + <i>Solanum Nigrum</i> (SN) Extract-Treated		
5. CCl ₄ + SN100	43.28 ± 0.66 ^{b*}	0.24 ± 0.55 ^{b*}
6. CCl ₄ + SN200	46.02 ± 0.54 ^{b*}	0.25 ± 0.90 ^{b*}
7. CCl ₄ + SN300	47.29 ± 0.33 ^{b*}	0.28 ± 0.76 ^{b*}

Results are expressed as mean ± SEM; n = 10

No significant difference was noted between groups 1–3

^aas compared between vehicle control (group 2) and toxin-treated (group 4),

^bas compared between toxin-treated (group 4) and toxin + antidote-treated (groups 5–7)

Level of significance *p < 0.001

Units: SDH - μg formazon formed/mg protein/15 min; ATPase - μmoles i.p. released/mg protein/30 min

et al. 2008, have reported a similar increase in body weight of thioacetamide-induced fibrotic mice after administration of *S. nigrum* extract [7].

Histopathological (H & E) examinations revealed severe cytoplasmic vacuolization and fatty degenerative changes in the liver of CCl₄-treated mice (Fig. 1 c). Earlier reports have confirmed the disruption of lattice nature of hepatocytes, degenerated nuclei, ballooning of liver cells, disintegrated central vein and fibrotic changes in CCl₄-treated animals [29]. However, administration of the plant extracts alongwith the hepatotoxin caused reversal of the histopathological alterations induced by the oral administration of the toxin in a dose-dependent manner (Fig. 1 d-f). Lin et al. 2008, have reported that administration of *S. nigrum* extract to CCl₄-intoxicated rats resulted in reduced incidence of hepatocyte swelling, lymphocyte infiltration and fibrous tissue proliferation [11].

The results of plant extract treatment on CCl₄-induced changes in activities of some liver enzymes are depicted in Table 2. Table 3 shows the effect of SN treatment on various hepatic marker enzymes in serum of mice. Results indicate a marked increase in activities of these enzymes after treatment with CCl₄ which was significantly and dose dependently mitigated by the administration of the plant extract. CCl₄ intoxication results in severe damage to hepatic tissue membranes results in leakage of these enzymes into circulation. [30]. Raju et al. 2003, have reported that ethanolic extract of *S. nigrum* possessed remarkable hepatoprotective activity against CCl₄-induced hepatic damage in rats by evaluating biochemical parameters such as AST, ALT, ALP and total bilirubin [8]. The histopathological changes of liver sample of extract-treated rats were normal and resembled control animals. Lee and Lim (2009) have investigated the potential of glycine and proline-rich glycoprotein isolated from *S. nigrum* in decreasing the leakage of LDH in 1, 2-dimethylhydrazine-treated mice [31].

In the present study carbon tetrachloride treatment for 30 days caused a significant and dose-dependent reduction in hepatic DNA, RNA and protein levels (Table 4). The Metabolic activation of CCl₄ results in production of free radicals which form covalent bonds with tissue macromolecules like DNA, RNA and proteins. It is well known that administration of CCl₄ results in dislocation of ribonucleoproteins from rough endoplasmic membranes causing an inhibition in protein synthesis [32]. Omar et al. 2006, have found out that CCl₄ to diminish the levels of DNA in liver by using Feulgen histochemical staining technique [33]. Voronova et al. 1976, have reported decrease in RNA content after CCl₄ administration [34]. Treatment of *S. nigrum* extract alongwith CCl₄ during the present study significantly and dose-dependently ameliorated the toxin-induced

changes in all these biochemical parameters and enhanced the levels of DNA, RNA and stabilizing the protein synthesizing function of the liver. The liver protecting property of Liv-52 which includes *S. nigrum* as a major ingredient against CCl₄-mediated liver damage was evaluated in rats by [35]. Their results revealed that CCl₄ caused significant reduction in the liver microsomal protein and RNA. However rats pre-treated with the indigenous drug Liv-52 and subjected to challenge with the hepatotoxin showed complete recovery.

Table 5 indicates the results of activities of SDH and ATPase of different doses of plant extract with and without CCl₄ treatment. Reduction in activity of SDH due to CCl₄ treatment indicates damage to mitochondria and hence results in overall reduction in energy status of the system [36]. Similarly uncoupling of oxidative phosphorylation and a decrease in ATPase activity due to CCl₄ treatment has also been reported [37]. Treatment with *S. nigrum* extract resulted in restoration of SDH and ATPase levels thus improving the energy status of the hepatic system.

The present study has reported a very potent hepatoprotective property of standardized flavonoid extract of *S. nigrum*. Flavonoids are considered to be the most frequently occurring polyphenolic Phytochemical with high medicinal properties. Many previous studies have reported the liver protecting property of *S. nigrum* against CCl₄-induced acute toxicity. In the present study *S. nigrum* was found to confer significant dose-dependent hepatoprotection against CCl₄-induced sub-chronic toxicity by improving the DNA, RNA and protein levels and also by increasing the energy levels of the hepatic tissue.

Conclusion

The present study has identified *S. nigrum* to be a potent hepatoprotective plant and it was found to confer hepatoprotection probably due to its high flavonoid content by preventing CCl₄-induced adduct formations in tissue macromolecules. More systematic studies are required to clearly understand the mechanistic aspect and also the molecular mechanism responsible for the potent hepatoprotective property of the plant *S. nigrum*.

Abbreviations

CCl₄: carbon tetrachloride; SN: Solanum nigrum

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Availability of data and materials

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Authors' contributions

KR designed the study, performed the experiments and acquired the data and drafted the manuscript; RJV designed and supervised the study; both authors critically revised and approved the manuscript.

Authors' information

Department of Zoology, Biomedical Technology and Human Genetics, University School of Sciences, Gujarat University, Ahmedabad – 380,009, India.

Ethics approval and consent to participate

The study was approved by "The Committee for the Purpose of Control and Supervision of Experiment on Animals" (Reg – 167/ 1999/ CPCSEA), New Delhi, India.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no conflicts of interest.

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