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ORIGINAL ARTICLE

Bioautography-based Identification of Antioxidant Metabolites of *Solanum nigrum* L. and Exploration Its Hepatoprotective Potential against D-Galactosamine-induced Hepatic Fibrosis in Rats

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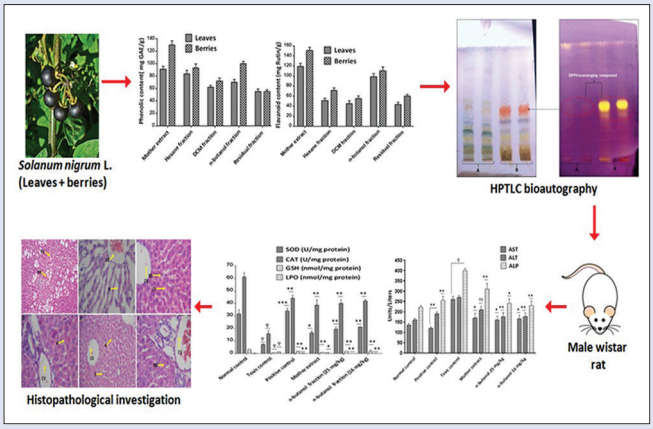
ABSTRACT

Objective: A traditional herb *Solanum nigrum* L. is well known for the management of different ailments including hepatic disorders. The objective of our study is to identify antioxidant metabolites and bioactive fraction of *S. nigrum* and to explore their hepatoprotective potential. **Materials and Methods:** The aerial parts (leaves and berries) of *S. nigrum* were extracted with hydroethanol- and polarity-based fractionations were performed. Total phenolic (TP), flavonoid content, and thin-layer chromatography (TLC) fingerprints of different extracts were carried out for their quality control and determination of compounds present in them. TLC-based bioautographic assay was carried out to identify the antioxidant metabolites. The hepatoprotective activity of a steroidal glycoalkaloid-enriched fraction of *S. nigrum* berries was investigated in D-galactosamine (D-GalN)-induced hepatic fibrosis. Hepatic damage was evaluated by assessing enzymatic activities of oxidative markers in serum and liver homogenate and histological study of the liver. **Results:** The n-butanol fraction of *S. nigrum* (berries) was found to have the highest value of TP and flavonoids. The treatment of rats with 250 mg/kg crude extract as well as 16 and 25 mg/kg of n-butanol fraction for 10 days was able to normalize the biochemical markers along with liver antioxidative markers in D-GalN treated hepatotoxic rats. The histopathological studies revealed that n-butanol fraction treatment also restored the markers of fibrosis toward a normal level. **Conclusion:** The n-butanol fraction from *S. nigrum* berries showed *in vitro* and *in vivo* hepatoprotective activity and can be explored after further investigations as a potent phytopharmaceuticals for the management of liver disorders.

Key words: Antioxidant, bioactivity, hepatoprotective, *Solanum nigrum,* steroidal glycoalkaloid

SUMMARY

- The hydro-alcoholic extract of leaves and berries and their steroidal glycoside enriched fraction of *Solanum nigrum* was prepared as n butanol fraction of berries. The mother extract and enriched fraction was found good hepatoprotective agent against in vivo galctosamine model in rats. Further, bioautographic identification showed solasodine as antioxidant compound.



Abbreviations used: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; CCl4: Carbon tetrachloride; CAT: Catalase; D-GalN: D-galactosamine; DCM: Dichloromethane; ECM: Extracellular matrix; FC: Folin–Ciocalteu; GSH: Glutathione; HSCs: Hepatic stellate cells; TLC: Thin-layer chromatography; LPO: Lipid peroxidation; NC: Normal control; PBS: Phosphate buffer saline; ROS: Reactive oxygen species; SOD: Superoxide dismutase; SNL: *Solanum nigrum* L.; STP: Serum total protein; SGAs: Steroidal glycoside alkaloidal; SOD: Superoxide dismutase.

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INTRODUCTION

The liver is the major site of xenobiotic metabolism and related to growth, development, and immunity. Hepatic fibrosis is considered as a self-healing response of the liver toward various chronic hepatic disorders along with hepatic injury and inflammations.[1] A variety of previous reports showed that the hepatic injury stimulates the hepatic stellate cells which produce extracellular matrix proteins (ECMs).[2] An imbalance between the production and utilization of ECMs leads to an excessive accumulation of ECMs which ultimately causes hepatic fibrosis.[3] In addition, reactive

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oxygen species (ROS) also play a critical role in the advancement of hepatic fibrosis causing hepatic cell damage and apoptosis.[4] The hepatic fibrosis may lead to cirrhosis which is an advanced stage of hepatic fibrosis with disruptive normal parenchyma and vascular structure.[5] However, various studies showed that the hepatic fibrosis and even cirrhosis are pathologically reversible and can be prevented in early stages.[6] In India, a number of natural compounds and their formulations are used for hepatic disorders. One of the most commonly used traditional plants is *Silybum marinum*. A number of well-designed experimental studies suggest that silymarin showed beneficial effects in chronic liver diseases. The popularity of herbal remedies is increasing and at least one-quarter of patients with liver disease use botanicals. Unfortunately, well-performed clinical trials on their therapeutic value in the treatment of certain liver diseases are very rare and the frequency in which herbal drugs cause hepatic damage remains uncharted since its use is largely uncontrolled.[7] Therefore, finding a safe and effective medicine that able to prevent hepatic fibrosis by reversing it is of unmet need.

Solanum nigrum L. (SNL) belongs to the nightshade of the family *Solanaceae* spread globally in the tropical as well as temperate regions.[8] The *Solanaceae* family are spread throughout the tropical as well as temperate regions of the world. Several types of glycoalkaloids especially steroidal glycosides have been isolated so far from *Solanum* species, and these have numerous benefits for human health as well as for ecological viewpoint. Previous phytochemical studies reveal that the medicinal activity of the plant is probably due to its flavonoid and steroidal glycoalkaloid content.[9] Varieties of functional groups such as hydroxyl, acetyl, sugar moieties present in steroidal glycoalkaloids and these are mainly responsible for biological activities.[10] *S. nigrum* has been used ethnobotanically as hepatoprotective, antipyretic, anti-inflammatory, antioxidant, diuretic, anticancer and microbial, activities. Previous phytochemical studies reveal that these medicinal activities are probably due to its flavonoid and steroidal glycoalkaloid content.[11] The glycoalkaloids (solanine, solamargine, solasonine, and solasodine) showed liver protective effects against CCl_4-induced liver damage. However, the aerial part (leaves and berries) of this plant have not been fully explored for their bioactivity guided fractionations and their metabolomic analysis. In our previous study, methods for simultaneous estimation of solamargine, solasonine, and solasodine through thin-layer chromatography (TLC) and by UPLC-ESI-MS/MS were reported.[12] In the present investigation, we identify the bioactive fractions from the aerial part and exploring their therapeutic potential in D-galactosamine (D-GalN)-induced hepatic fibrosis in rats. Thus, the present study explored the chemically characterized bioactive extract of *S. nigrum* for the evaluation of hepatoprotective potential.

MATERIALS AND METHODS

Materials

Aerial parts (leaves and ripen berries) of SNL were collected from the local fields of Aligarh (India) and was authenticated as per protocol mentioned in Ayurved Pharmacopoeia. The specimen has been deposited in Bioactive Natural Product Laboratory (BNPL), Jamia Hamdard, New Delhi (Specimen No.: JH/FP/BNPL/2014/Karishma/S1) for future reference.

2, 2-diphenyl-1-picrylhydrazyl (DPPH, 0.002%, w/v) and additional assays kits were purchased from Accurex Biomedical kits (Mumbai, India). Silica gel 60F_{254} plates were purchased from Merck (Darmstadt, Germany). D-GalN, Folin–Ciocalteu's (FCs) reagent, phenol, and gallic acid were purchased from Sigma Aldrich (USA). Phosphate buffer saline (PBS) was purchased from Gibco®, Thermo Fisher (USA). All other chemicals used during the experiment were of analytical grade and procured from Merck Ltd., India.

Preparation of hydroalcoholic extract (crude extract) and its fractions

The amount of powdered *S. nigrum* aerial parts (500 g, leaves and berries) were dried in the shade and extracted using ethanol (70% v/v) for 5 h using reflux extractor. After filtration, the extract was evaporated to dryness in vacuo (vacuum degree-0.1 MPa) at 60°C. The extract prepared from both samples (leaves and berries) were collected separately. The hydroalcoholic extracts of *S. nigrum* leaves and berries were suspended in double distilled water (1 g/10 mL) and sonicated for 15 min at 45°C. The aqueous solution of crude extract was fractionated using organic solvents in increasing order of polarity (n-hexane, dichloromethane [DCM], and n-butanol) and the remaining portions were collected as aqueous fraction. The fractions obtained were evaporated to dryness in vacuo. The extractive values and % yields of extract and their different fractions were determined, and the fractions were stored at 4°C for future studies.

Determination of total phenolic and flavonoid content

FC assay and aluminum chloride colorimetric method were used for the determination of total phenolic (TP) and total flavonoids (TF) content of various fractions of *S. nigrum*.[13,14] Different concentrations of standard (gallic acid and rutin) were used for preparing the calibration curve used for calculating TP and flavonoid content. The results of TP and flavonoid content were expressed as mg of gallic acid and rutin equivalent to per gram dry weight of the extract, respectively.

Thin-layer chromatography-bioautography-2, 2-diphenyl-1-picrylhydrazyl of extract and fraction for antioxidant activity

TLC fingerprints of different extracts were carried out for their quality control and determination of a number of compounds present in them.[15] *S. nigrum* extract and fractions (100 g) were reconstituted in the volumetric flasks using methanol to get a final solution concentration of 10.0 mg/mL. As a mobile phase, n-butanol_ethyl acetate_acetic acid (5:3.5:1.5, v/v/v) was used. The developed plates were dried, derivatized in anisaldehyde-sulfuric acid reagent and were scanned at 540 nm. The samples and the standards (2.0 µL each) were applied on a precoated silica gel 60 F_{254} aluminum TLC plate (MERK, Germany) using Linomat V applicator (CAMAG, Switzerland). The applied plates were developed up to 80 mm in twin trough glass chamber saturated with the respective solvent system.

In order to identify the antioxidant metabolites present in bioactive fractions, TLC bioautography technique was used.[16] The same mobile phase was utilized for bioautography analysis. The developed underivatized plate was dried and dipped in 0.5 mM DPPH solution in ethanol. Compounds having free radical scavenging activity were determined *in situ* using DPPH reagent (2.54 mM).[17] The TLC plates were observed under visible light, and the bands which changed their color from purple to yellow against the purple background were considered as antioxidant metabolites. Further, the compound was isolated and identified by mass spectrometry. In addition, densitometric scanning was performed on CAMAG TLC scanner in the absorption mode at 540 nm using the same slit dimension 5.00 mm × 0.30 mm.

In vivo hepatoprotective activity

Animals and treatments

Hepatoprotective potential of the n-butanol fraction of *S. nigrum* berries against D-GalN (D-GalN)-induced hepatic fibrosis was studied in 10-week-old male Wistar rats (weighing 200–250 g). The experimental

procedures and protocols were approved by the Institutional Animal Ethical Committee as per the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals, Central Animal House Facility of Hamdard University (Approval No: 1017). The rats were acclimatized for a week under standard housing with controlled conditions (25°C ± 2°C, 60% ± 10% humidity, with a 12 h light-dark cycle).

After 1 week of acclimatization, rats were randomly divided into five groups with six animals in each group. Group I has received a normal diet with water *ad libitum*, served as control. Animals of Group II-V were treated with D-GalN (400 mg/kg, i. p). Apart from D-GalN treatment, animals of Group II received normal saline for 10 days as drug and served as a toxic group. Group III received silymarin (25 mg/kg, orally for 10 days) served as positive control. Group IV was treated with a crude extract of berries (250 mg/kg, orally for 10 days). Group V and VI received a steroidal enriched fraction of berries at a dose of 25 and 16 mg/kg for 10 days, respectively. To determine whether the hepatic fibrosis model was successfully established, liver biopsy was conducted, and the rats with hepatic fibrosis were selected for further studies.

Sample collection

After 24 h of the treatment with D-GalN, animals were anesthetized by inhalation of ethyl ether and blood was collected. The blood sample was allowed to clot, and serum was separated by centrifugation at 3000 rpm for 15 min. The serum was then used for the estimation of the biochemical parameters. Following the blood sample collection, rats in all groups were sacrificed, and the weights of the liver were measured. Liver samples were collected for histological and biochemical estimations. The liver samples collected were washed with normal saline, and liver homogenate (10% w/v) was prepared for the analysis. Some samples were stored at −80°C for further studies. Soon after the sample collection, a part of the liver tissue was transferred into 10% formalin for histopathological investigation.

Measurement of serum biochemical markers

The blood samples were allowed to clot at 4°C and then centrifuged at 3000 rpm for 10 min at 4°C to obtain the serum. The level of hepatic damage was evaluated by analyzing enzymatic activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and serum total protein.[18] The enzymatic activities were determined by using commercially available kits (Accurex Biomedical kits, Mumbai, India).

Measurement of glutathione, catalase, superoxide dismutase, and lipid peroxidation in homogenized liver tissues

The hepatic tissues were homogenized in ice-cold PBS (50 mM, pH 7.4). The resulting suspension was centrifuged at 4000 rpm for 10 min at 4°C, and the supernatant was collected for further analysis. The activities of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and lipid peroxidation (LPO) were assayed using commercially available kits (Accurex Biomedical kits, Mumbai, India).[19]

Histological examination

Fresh liver slices were fixed with formalin (10%) in PBS for 24 h and embedded in paraffin. A thick section (4 μM) was prepared using microtome and stained with h and e. The stained sections were analyzed at ×400 magnification to observe histopathological changes in the liver.[19]

Statistical analysis

The statistical analysis was performed using Graph Pad Prism 5.0.1 (Graphpad Software Inc, San Diego, CA, USA). To evaluate the significant differences between all the parameters in respective treatment groups, one-way ANOVA followed by Dunnett's test was performed. The data were expressed as mean ± standard deviation and $P < 0.05$ and $P < 0.01$ were considered to be statistically significant.

RESULTS

The crude extract of leaves and berries was subjected to polarity-based fractionation using n-hexane, DCM, and n-butanol. The yield (%) of crude extract and fractions was found higher in berries compared to leaves [Figure 1a]. The TP content (mg GAE/g) and TF (mg rutin/g) were found higher in berries compared to leaves [Figure 1b and c].

The simultaneous determination of bioactive compounds is a challenging task due to the matrix complexity and variation of the components. To date, few methods have been developed for the quantification of steroidal glycosides, such as solamargine, solasonine, and solasodine.[20-22] Therefore, in the previous study, we have reported novel, rapid and sensitive TLC and UPLC-ESI-MS/MS methods for the simultaneous estimation of solamargine, solasonine, and solasodine.[11] In continuation, the present investigation deals with the evaluation of the antioxidant and hepatoprotective effect of a steroidal glycoalkaloid enriched fraction of SNL against D-GalN-induced hepatic fibrosis in rats.

Thin-layer chromatography fingerprinting

For TLC fingerprinting, different compositions of solvent systems were tried to obtain good resolution bands and reproducible peaks. The desired mobile phase was attained using n-butanol_ethyl acetate_acetic acid (10:7:3, v/v/v). TLC fingerprinting profile of *S. nigrum* extract and fraction contents has been shown in Table 1.

Thin-layer chromatography bioautography for antioxidant activity

To screen the antioxidant capacity of n-butanol fraction of *S. nigrum* aerial parts (leaves and berries), a TLC bioautography method was performed. The TLC plate treated with the DPPH reagent showed antioxidant compounds as a yellow or golden spot on a purple background [Figure 2a and b]. TLC derivatized plate dipped with 2.54 mM DPPH showed yellowish bands on the purple background were considered having antioxidants. In mass spectrometry, m/z of the compound was found 414.56, and thus, the antioxidant metabolite was solasodine [Figure 2c].

Assessment of liver function markers

The hepatoprotective effect of the crude extract (250 mg/kg by weight) and steroidal glycoside containing n-butanol fraction (25 and 16 mg/kg by weight) on the enzymatic activities of serum ALT, AST, and ALP were evaluated. In the normal group, serum ALT, AST, and ALP levels were 122.4 ± 6.5, 150.7 ± 154, and 198 ± 6.5 IU/L, respectively [Figure 3]. After the induction of D-GalN, the serum ALT, AST, and ALP activities showed significant increases 259 ± 22.7 ($P < 0.01$), 289 ± 25.4, and 441 ± 25.4 IU/L ($P < 0.01$), respectively. The crude extract (250 mg/kg) and n-butanol fraction containing steroidal glycoside (25 and 16 mg/kg) showed similar effects on the serum total bilirubin (STB). Compared to the normal group (0.94 ± 0.12 mg/dL), ($P < 0.01$), the D-GalN-induced rats STB was sharply increased by 3.0-fold i.e., (3.11 ± 0.15 mg/dL) [Figure 4]. Pretreatment with the n-butanol fraction reduced the STB levels increased by the D-GalN treatment. At a dosage of 16 mg/kg per weight, STB levels reduced to 0.81 ± 0.15 mg/dL [Figure 4].

Table 1: Thin-layer chromatography fingerprint profile of crude extract and different fractions of *Solanum nigrum* (leaves and berries)

R_f/compound	Leaves					Berries				
	Crude extract	Hexane fraction	DCM fraction	*n*-butanol fraction	Aqueous fraction	Crude extract	Hexane fraction	DCM fraction	*n*-butanol fraction	Aqueous fraction
0.11 (solasonine)	+	–	+	+	–	+	–	+	+	–
0.17	+	–	–	+	+	+	+	–	+	+
0.22 (solamargine)	+	–	+	+	–	+	–	+	+	–
0.24	–	–	–	–	+	+	–	–	–	+
0.34	+	+	–	–	–	+	+	+	–	–
0.37	+	–	+	–	–	+	–	+	–	–
0.43	+	–	+	–	+	+	+	–	–	+
0.46	+	+	–	–	–	–	–	+	–	+
0.52	+	+	+	+	–	+	–	+	+	–
0.60	+	–	+	+	+	+	–	–	+	+
0.63 (solasodine)	+	–	+	+	+	+	–	–	+	+
0.7	+	–	+	–	+	+	–	–	–	+
0.75	+	–	–	+	+	+	–	+	–	+

DCM: Dichloromethane. – absent; + present

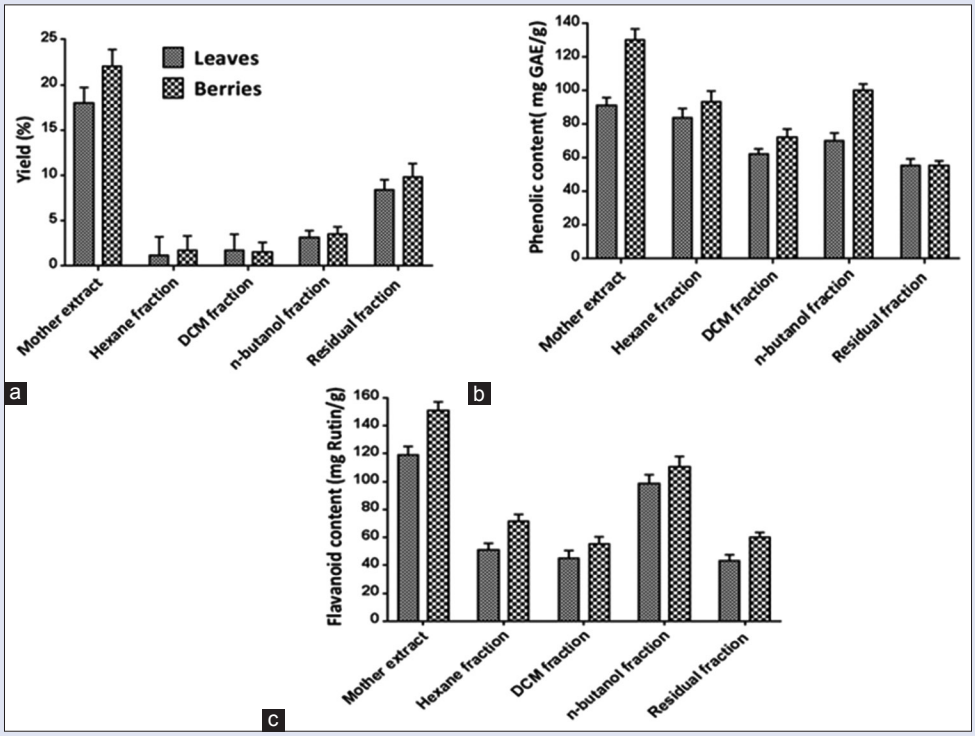

Figure 1: Total phenolic and flavonoid content, (a) percentage yield, (b) total phenolic and (c) total flavonoid content of the fractions of *Solanum nigrum* L. (leaves and berries)

Hepatic antioxidant markers

The crude extract (250 mg/kg by weight) of *S. nigrum* and its n-butanol fraction (25 and 16 mg/kg by weight) and were administered once daily for 10 consecutive days before the single administration of D-GalN. Results showed a significant decrease in hepatic GSH, SOD, and CAT which considered as an index of the antioxidant status of tissues. There was a significant increase in these antioxidant enzyme activities in n-butanol fraction ($P < 0.001$) and crude extract ($P < 0.01$) treated rats compared to the D-GalN only treatment [Figure 5].

Histopathological investigation of rat liver

Histopathological observation is a key contributor to provide a supportive evidence for biochemical analysis. The histological findings were found in coherence with the biochemical results [Figure 6]. The liver section in normal control rats showed the typical hepatic architecture, such as central vein surrounded by a hepatic cord of cells [Figure 6a]. Liver sections of D-GalN intoxicated rats showed congestion in a central vein, necrosis, and the loss of cellular boundaries [Figure 6b]. Interestingly, the administration of n-butanol fraction and crude extract showed protective action against D-GalN-induced liver damage [Figure 6c and d]. The hepatoprotective property of *S. nigrum* extract could be due to the antioxidant property of the major constituents of the plant.

DISCUSSION

The herbal metabolites and derived compounds are widely used worldwide due to their efficacy with fewer side effects. The liver is a vital organ of the body that regulates important processes related to human physiology

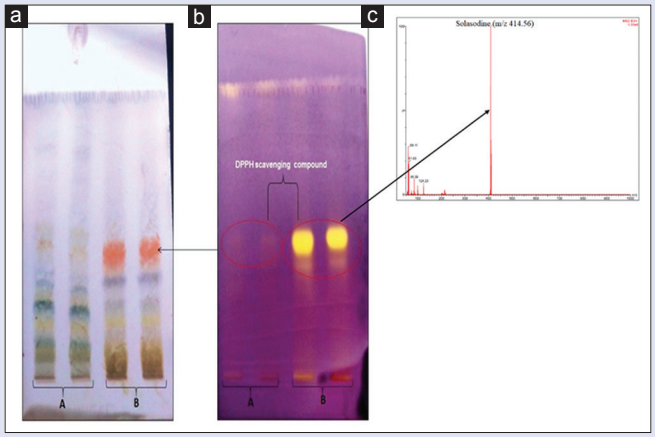

Figure 2: Thin-layer chromatography plate of n-butanol fraction of crude extract of leaves and berries chromatographed using n-butanol: ethyl acetate: acetic acid, (5:3.5:1.5, v/v/v) visualized after (a) anisaldehyde derivatization, (b) underivatized plate dipped with 2.54 mM 2, 2-diphenyl-1-picrylhydrazyl, yellow spot against purple background showing antioxidant metabolites, and (c) solasodine was identified as antioxidant metabolite

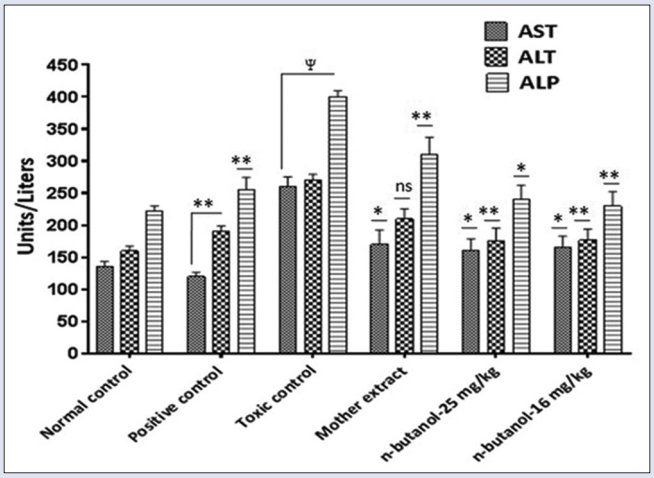

Figure 3: Effects of steroidal enriched fraction on liver function markers. Data were presented as mean ± standard error of the mean (n = 6), $^{\Psi}P < 0.01$ when toxic control compare with control, ns: Non-significance $^{*}P < 0.05$, $^{**}P < 0.01$ versus toxic control

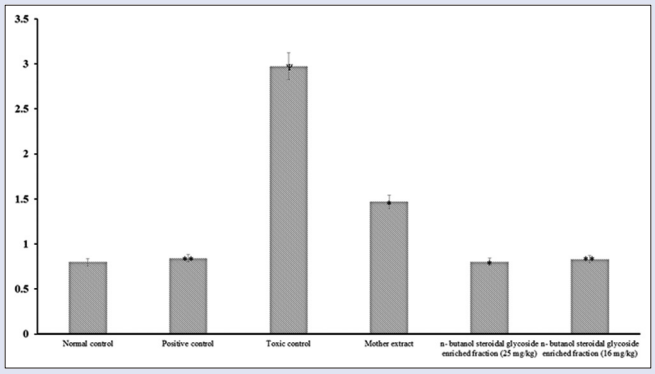

Figure 4: Effects of steroidal enriched fraction on total bilirubin in hepatic serum. Data were presented as mean ± standard error of the mean (n = 6), $^{\Psi}P < 0.01$ when toxic control compare with control, $^{*}P < 0.05$, $^{**}P < 0.01$ versus toxic control. Data were presented as mean ± standard deviation, number of animals (n) =6

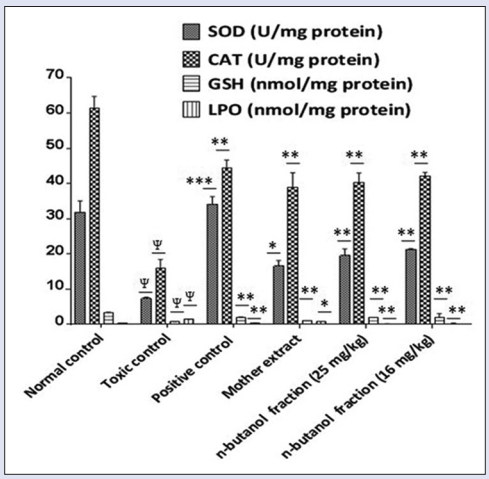

Figure 5: The effects in levels of hepatic antioxidant markers (superoxide dismutase, catalase, glutathione and lipid peroxidation) in liver tissue of control and experimental rats on drug administration. Each value is represented as mean ± standard error of the mean, number of animals (n) = 6, $^{\Psi}P < 0.01$ when toxic control compare with control, ns: Non-significance $^{*}P < 0.05$, $^{**}P < 0.01$ versus toxic control

and metabolisms. Liver is exposed to various drugs and xenobiotics thus prone to toxicity. Today's lifestyle is also an important factor that contributes toward widespread hepatic disorders. To date, a number of natural extracts, pure compounds and derived products have been reported for effective management of hepatic diseases.[23] SNL is widely used as an anticancer, immunomodulator, antimicrobial, nematicidal, antioxidant, anticonvulsant, hepatoprotective, antiulcerogenic, and anti-inflammatory agent.[24]

In spite of the remarkable therapeutic potential of *S. nigrum*, its chemical composition is still unknown. Therefore, we carried out a polarity-based fractionation process that leads to identify the key therapeutic candidate from a phytochemical matrix. *S. nigrum* contains various compounds (glycoalkaloids, glycoproteins, and polysaccharides) responsible for various therapeutic effects. The glycoalkaloids include tropane type compounds, such as solamargine, solasonine, and solasodine.[24] The study showed that steroidal glycosides are present in higher quantity in the n-butanol fraction of berries compared to leaves [Figure 1]. TLC fingerprinting is an important quality control tool for herbal samples. In the present study, TLC fingerprinting profile of *S. nigrum* crude extract and fractions of leave and berries were evaluated which showed different phytochemical constituents solasonine, solamargine, and solasodine [Table 1]. There are some metabolites present at different R_f such as at 0.24 (in leaves) and 0.46 (in berries) of fractions but not in crude extract. There are some metabolites which appearing at particular R_f in crude extract were also found present in different in opposite fraction polarity (e.g., R_f 0.43 of berries, 0.60 and 0.43 of leaves), which means that these metabolites are not same but different and appeared at that particular R_f due to use concentrated samples. However, the presence of metabolites at same R_f does not necessarily qualify that these metabolites are same, but it may be different and appeared in TLC at the same R_f due to the use of concentrated samples.

TLC bioautography assay was used to determine the active antioxidant in a mixture of compounds as it is a simple and flexible method with high

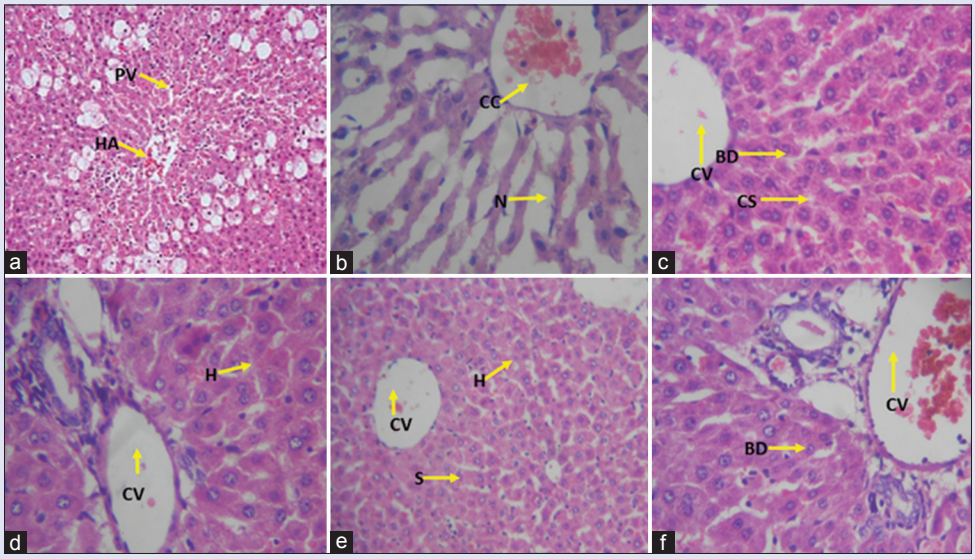

Figure 6: Hepatoprotective potential of *Solanum nigrum*. Rat of (a) normal control group; (b): Toxic control group; (c): Standard drug (Silymarin) group; (d) *n*-butanol fraction *Solanum nigrum* berries (25 mg/kg), (e) *n*-butanol fraction *Solanum nigrum* berries (16 mg/kg) and (f) crude extract of *Solanum nigrum* berries (250 mg/kg). CV: Central vein, PV: Portal vein, HA, N: Necrosis, H: Hepatocytes, MN: Mild necrosis, CS: Congestion in sinusoidal spaces, BD: Ballooning degeneration, CC: Congestion in central vein and S (sinusoids)

throughput. On the TLC plates, antioxidant compounds were seen as yellow spots on a purple background [Figure 2]. Based on the mass and charge ratio obtained from mass spectrometry analysis of compound, the antioxidant metabolites were identified as solasodine. Moreover, phenolic and flavonoid contents of the crude extract as well as its fractions were measured. Crude extract and n-butanol fractions contained significantly higher amount of phenol and flavonoid constituents compared to other fractions. Similarly, the same pattern in antioxidant activity was obtained for crude extract and fractions. Thus, this study contributes to the understanding of the strong positive relationship between the TP or flavonoid content and the antioxidant activities.[24]

The hepatoprotective potential of the crude extract and an n-butanol fraction of *S. nigrum* were studied on D-GalN induced hepatic fibrosis in rats. The excessive production of free radicals leads to oxidative stress followed by hepatic toxicity.[25] Thus, the extracts possessing free radical scavenging and/or antioxidant activities could also demonstrate hepatoprotective activity. Studies have shown that the combination of hepatoprotective and antioxidant activity synergistically prevents the process of initiation and progress of hepatocellular damage. The hepatic toxicity also results in the disruption of cell membrane followed by the enzyme leakage. This results to an increase of the serum level of ALT, AST, and ALP enzymes in D-GalN-treated rats. The n-butanol fraction successfully reversed the negative impact of D-GalN on the liver and decreased the serum level of the enzymes (ALT, AST, and ALP) [Figure 3]. The crude extract (250 mg/kg) and n-butanol fraction enriched with steroidal glycosides (25 and 16 mg/kg) showed similar effects on the STB (TB) [Figure 4].

One of the essential parameters for oxidative stress is the LPO.[26] The n-butanol fraction showed a reduction in the lipid peroxidase (LPO) level which indicates the free radical scavenging activity of *S. nigrum* extract under *in vivo* conditions. GSH is considered as a first line defense against free radicals and play a crucial role in managing the antioxidant defense processes in the body.[27] Under oxidative stress conditions, GSH transforms into GSH disulfide (GSSG) causing LPO. The present study demonstrated that the D-GalN treated rat liver showed a significant reduction in the activity of antioxidant enzymes (CAT, SOD, GSH, and GST). These enzymes provide protection against harmful effects ROS and free radicals in biological systems. SOD is a scavenging agent of free radicals, and GSH is a reducing agent of H_2O_2. In addition, GSH protects the integrity of cell membranes by maintaining antioxidant defense mechanism conjugates with free radicals. The reduction of these enzyme activities is due to the activation of protein levels by ROS.[28] The treatment of n-butanol fraction reversed the process and resulted in an increase of the antioxidant enzymes activity. In addition to the oxidative stress, the antioxidant enzyme SOD and CAT also protect the tissue damage [Figure 5].[26]

The histopathological observation validates the findings of the biochemical analysis. D-GalN induced hepatic fibrosis in rats caused damage to the hepatic architecture and produced histological changes such as vacuole formation, neutrophil infiltration, and necrosis of hepatocytes. Pretreatment with the crude extracts (250 mg/kg) and their n-butanol fractions (16 and 25 mg/kg) resulted in a significant improvement in the structure of hepatic cells [Figure 6]. Overall, histopathological observation results showed a close relationship with the results detected in case of biochemical parameters.

CONCLUSION

Our results showed that administration of n-butanol fraction of SNL (leaves and berries) impart significant hepatoprotective effect. The effects of the n-butanol fractions are probably due to their free radical-scavenging and antioxidant properties. The hepatoprotective effect is also evident through histological studies of D-GalN-induced hepatic fibrosis in rats. Further, molecular evaluation needs to be performed for defining the probable mechanism behind the hepatoprotective activity. However, an n-butanol fraction of *S. nigrum* can be explored for the development of new phytopharmaceuticals for the management of liver disorders.

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Conflicts of interest

There are no conflicts of interest.

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