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The effects of bioactive components in *Solanum nigrum* against oxidative stress in liver damage

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Abstract

The present study aimed to evaluate the role of bioactive components in *Solanum nigrum* fruit, its phytochemical screening, and in vivo antioxidant potential *against liver injury.* The HPLC analysis of an ethanolic extract of *Solanum nigrum* fruit revealed the presence of bioactive components that showed its significant in vitro antioxidant activity. Moreover, The Sprague Dawley rats were divided into four groups for in vivo analysis: G1 (negative control), G2 (positive control), G3 (rats receiving standard drug), and G4 (rats receiving *Solanum nigrum* fruit extract). The *Solanum nigrum* fruit reduced the level of liver enzymes, and bilirubin. An opposite trend was seen in the case of albumin, catalase, creatinine, and superoxide dismutase. Histology slides also showed the normal cell structure of hepatocytes. Conclusively, *Solanum nigrum* fruit bioactive components have the ability to reduce oxidative stress.

Keywords: bioactive components; antioxidant; *Solanum nigrum*; liver; oxidative stress.

Practical Application: It is stated that bioactive ingredients which are present in antioxidant rich *S. nigrum* fruit can be suggested to add in not only to personalized nutrition but also in community nutrition to combat health-related problems like acute liver toxicity.

1 Introduction

S. nigrum is a herb belongs to the Solanaceae family and includes in a class of Dicotyledonae. *S. nigrum* is also known as black nightshade, garden nightshade, or blackberry nightshade (Zaidi et al., 2019). In most of the cases, it grows in the form of weeds in habitats which are moist in nature. It also has capability to grow in stony, deep or dry soils, their seeds are best grows in the months of April-May (Teng et al., 2022b). *S. nigrum* consist of many constitutes like anthocyanins, anthocyadins, flavonoids, tanins, vitamin C, vitamin E, and small quantities of iron, zinc, selenium, gallic acid and quercetin which act as an active ingredient in controlling oxidation which results in the prevention of oxidative stress, acute liver toxicity and metabolic ailments such as diabetes, cardiovascular diseases and many types of cancer (Saibu et al., 2020). *S. nigrum* is one of the richest sources of anthocyanins after berries (Huang et al., 2022; Li et al., 2021a). Being antioxidants in nature, they donate the pair of electrons and stabilize the free radicals in body (Khan et al., 2021; Teng et al., 2022a).

The role of *S. nigrum* is significant against acute liver toxicity due to presence of active ingredients (Aabideen et al., 2022). Out of all the parts, leaves and fleshy portion of *S. nigrum* are used mostly for therapeutic functions (Campisi et al., 2019). *S. nigrum* has the ability to act as an anti-tuberculosis, anti-viral, anti-oxidant and anti-inflammatory agent. Antioxidant ability of *S. nigrum* is described by previous investigations, which prevents free radical oxygen species in hepatotoxicity (Parveen et al., 2020). About 2 million deaths per year occur due to liver toxicity worldwide and 60% of them have acute toxicity (Xiao et al., 2022).

In an experimental design, phenylhydrazine has the ability to form reactive molecules like oxygen radicals and superoxide anions. These products causes the not only the lipid peroxidation but also the damage to membrane (Okafor & Atsu, 2022). All the liver enzymes and bilirubin values were increased with the intake administration of single dose of phenylhydrazine, which in return cases the lipid peroxidation (Souza et al., 2022). In light of the aforementioned facts, the purpose of the study is to evaluate the antioxidant potential and phytochemical characterization of *S. nigrum* using HPLC analysis. Furthermore, the hepatoprotective effect of *S. nigrum* extract against phenylhydrazine induced acute liver toxicity was assessed in Sprague Dawley rats.

2 Material and methods

2.1 S. nigrum extract preparation

For ethanolic extraction of *S. nigrum* fruit, a protocol by Iwansyah et al. (2021) and Sasidharan et al. (2011) was used, 10 g sample was taken. Each sample was mixed with 60% ethanol to make a solution up to 100 mL. After that, the samples were subjected to an orbital shaker (KS-260 Edmund Buhler Gmg

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H-Ks 15) at 280 rpm for 3 h at a constant temperature of 30 °C. After shaking, the samples were centrifuged (MPW-352R) at 2500 rpm at 30 °C for about 10 min. followed by filtration of supernatant (solution containing precipitation). In the end, the solvent was evaporated using the EYELA Rotary evaporator.

2.2 High Performance Liquid Chromatography (HPLC) quantification of phenolic compounds

The phenolic acids in *S. nigrum* fruit were measured using the methodology of Chang et al. (2017) and Younas et al. (2020). A Shim-Pack CLC-ODS (C-18) column (5 m, 25 cm 4.6 mm) was included in this experiment. A UV-Vis detector (SPD-10 AV) with a wavelength between 210-400 nm was used in the analysis. For the gradient elusion, A (H2O: acetic acid-94:6) and B (H2O: acetic acid-94:6) were utilized as mobile phases (100% acetonitrile). With a sample injection volume of 5 L, a flow rate of 1 mL/min was maintained. A standard chromatogram has been used to determine the phenolic acids in *S. nigrum* fruit samples depending on the relative retention time. The peak regions of each phenolic acid were evaluated as well as the results were transformed to g/mg.

2.3 In vivo study

Sprague Dawley rats weighting the range of 200 to 250 g b.w., were purchased from the animal house of University of Agriculture, Faisalabad (UAF), Pakistan, for an experimental trial. The experimental trial was carried according to guidelines of the National Biosafety Committee 2005, Punjab Biosafety Rules 2014, Punjab Animal ACT 2019, and Bioethical Protocols. The study was approved by Institutional Biosafety and Bioethical Committee (IBC, Ethical Issue No. 1315 University of Agriculture Faisalabad, Pakistan.

2.4 Treatment plan

Experimental protocols: Forty (40) Sprague Dawley rats were randomly distributed into 4 groups (n = 10).

- **Group 1 (G1):** Negative control group which remained untreated
- **Group 2 (G2):** Positive control group: Phenylhydrazine (65 mg/kg b.w. for 8 days) administrated rats (for the induction of acute liver toxicity) (Pandey et al., 2014)
- **Group 3 (G3):** Phenylhydrazine administrated rats with addition to the standard drug (silymarin)
- **Group 4 (G4):** Phenylhydrazine administrated rats with *S. nigrum* fruit extract (1.0 g/kg b.w.) for 30 consecutive days with the help of gavage.

At the end of trial, the blood was collected from the rats of all the groups from jugular vein.

2.5 Alanine Aminotransferase (ALT), Aspartate Transaminase (AST), Alkaline Phosphatase (AP)

For the estimation of ALT and AST, Reitman & Frankel (1957) method was used. AP level was assessed by using the protocols (King & King, 1954). Spectrophotometer (PG instruments, T80) was used to estimate the enzyme activity.

2.6 Serum bilirubin, creatinine and albumin

Serum bilirubin was estimated by following the method of Walters & Gerarde (1970). A commercial kit (Biotechnical; Varginha, Minas Gerais, Brazil) was used to estimate the amount of creatinine in Dawley rats. It was calculated by following the method of Rodrigues et al. (2014). The colorimetric method was used for the assessment of albumin in rats. For that purpose Gornall et al. (1949) method was used.

2.7 Superoxide Dismutase (SOD) and Catalase (CAT)

The activity of superoxide dismutase was found through a spectrophotometer (PG instruments, T80) as described by Abdel-Latif et al. (2020). The catalase activity in the liver tissues of rats was assessed by the method described (Krishnamoorthy & Sankaran, 2016).

2.8 Histological examination

The process includes fixation, dehydration, clearing, blocking out, embedding followed by microtome, mounting and sectioning. A microtome was used to slice the thick sections (5 µm) of liver tissues on glass slides. After that, process of staining was done by using hematoxylin-eosin and periodic acid- Schiff (Chen et al., 2020). The images of the slides were taken by using the light microscope (MCX 100, Micros Austria).

2.9 Statistical analysis

All the data were statistically studied by two-way ANOVA and reported as mean ± SE followed by Tuckey's HSD test. Values were considered significant when $p < 0.05$.

3 Results and discussion

3.1 Quantification of polyphenols

Polyphenols compounds present in *S. nigrum* fruit are presented in Table 1 i.e. gallic acid, benzoic acid, quercitrin, ferulic acid and caffeic acid. According to Peng et al. (2020), good antioxidant activities exhibited by plants extracts were

Table 1. Phenolic compounds present in *S. nigrum* fruit.

Phenolic Compounds	Concentration (µg/mg)
Gallic acid	6.02 ± 0.06
Benzoic acid	0.37 ± 0.03
Quercetin	1.23 ± 0.01
Ferulic acid	0.18 ± 1.16
Caffeic acid	0.08 ± 0.01

Values are mean ± SD for 3 determinations.

due to the presence of poly-phenolic compound. Chang et al. (2017) and Seon et al. (2021) reported that the main phenolic compounds found in the extract of *S. nigrum* were gallic acid, ρ-coumaric and caffeic acid. It also includes rutin, gossypin, and epicatechin. Hari et al. (2013) and Peng et al. (2020) conducted a study for the identification of phenolic compounds in reducing weight and body fat. In Huang et al. (2010) the phenolic extract was used to treat and prevent hepatocarcinoma. According to this study, this herb extract includes protocatechuic acid, gallocatechin and caffeic acid with the recovery time of 4.55%, 1.37% and 7.17% respectively.

3.2 Effect of S. nigrum on Aspartate Transaminase (AST), Alanine Transaminase (ALT), and Alkaline Phosphatase (AP)

Antioxidant potential of *S. nigrum* fruit was checked by estimating the fluctuations in liver enzymes i.e. aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (AP). They are chief indicator of hepatic injury and fluctuations occur in the case of hepatotoxicity. Statistical data showed that all the treatments have significant effect on the AST, ALT & AP (Figure 1a-1c). The level of all the liver enzymes were increased in phenylhydrazine administrated rats. Lowest AST and ALT level was noted in the G4 i.e. 215.75 ± 7.74 IU/L and i.e.92.13 ± 3.41 IU/L respectively where *S. nigrum* intervention was given to rats (Figure 1). The G2 had the highest AST and ALT levels, at 241.557.74 IU/L and 108.771.89 IU/L, respectively. AP is also one of the important indicators of liver, whom malfunctioning leads towards liver damage. Elevation of values can be seen in G2 i.e.645.63 ± 9.33 IU/L. G4 demonstrates reduced value i.e. 547.47 ± 0.38 IU/L as compared to G3 values i.e. 556.63 ± 0.31 IU/L (Figure 1c). Results from the study of Sheth et al. (2021) reported that increase level of liver enzymes (ALT, AST, and AP) is responsible for the progression of liver toxicity. Liver toxicity causes lipid peroxidation and resulted in the production of Reactive Oxygen Species (ROS) (Birben et al., 2012). The alterations in liver enzymes (ALT, AST and AP) upon administration of phenylhydrazine were also assessed by Ibrahim et al. (2018).

Values are mean ± SD significant difference between control and *S. nigrum*- treated dawley rats by t-test; *P < 0.01Unit: AST(IU/L), ALT (IU/L), AP (IU/L). G1: control group which remained untreated G2: phenylhydrazine administrated rats G3: phenylhydrazine administrated rats with addition to standard drug G 4: phenylhydrazine administrated rats with *S. nigrum* fruit extract (1.0 g/kg b.w.)

Different superscripts letters in the graph differ significantly (P < 0.01).

3.3 Effect of S. nigrum on bilirubin, albumin and creatinine

Bilirubin, albumin & creatinine were assessed to evaluate the potential of *S. nigrum* fruit in Sprague Dawley rats (Figure 2). Highest value of bilirubin was present in G2 i.e. 3.4600 ± 0.11 µmol/L (Figure 2a). G4 showed decreased bilirubin level (2.93 ± 0.1 µmol/L, P < 0.01) as compared to the positive control. This result also corresponds with the study of Salman et al. (2018) where bilirubin level was reduced to 0.26 ± 0.16 mg/dL from 0.56 ± 0.06 mg/dL. Low values of albumin and creatinine were present in phenylhydrazine treated rats. Albumin and

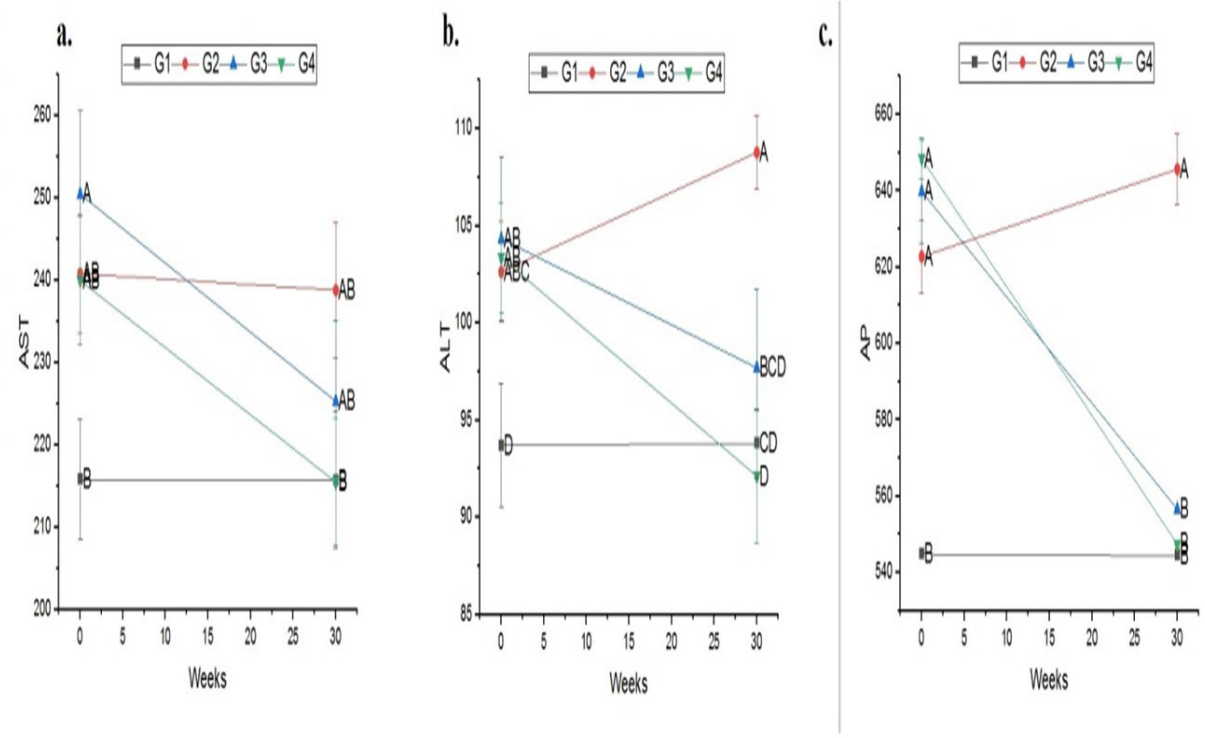

Figure 1. Effect of *S. nigrum* fruit on liver enzymes AST (a), ALT (b) & AP (c).

creatinine showed the values of 36.650 ± 1.36 g/L and 1.38 ± 1.08C µmol/L respectively in G4 which is higher as compared to G2 (Figure 2b-2c). This results also corresponds to the values of Salama et al. (2019) where hydro alcoholic extract of *S. nigrum* was given to glycol induced toxicity. Veerapagu et al. (2018) investigated that low level of albumin and creatinine in the phenylhydrazine-treated group is due to fibrosis leading to cirrhosis and the colloid osmotic pressure was also decreased.

Values are mean ± SD significant difference between Control and *S. nigrum*- treated Dawley rats by t-test; *P < 0.01.

Unit: Bilirubin (µmol/L), Albumin (g/L), Creatinine (µmol/L).). G1: control group which remained untreated G2: phenylhydrazine administrated rats G3: phenylhydrazine administrated rats with addition to standard drug G4: phenylhydrazine administrated rats with *S. nigrum* fruit extract (1.0 g/kg b.w.)

Different superscripts letters in the graph differ significantly (P < 0.01).

3.4 Effect of S. nigrum fruit on Catalase (CAT) and Superoxide Dismutase (SOD)

Figure 3 revealed that the highest value of catalase (CAT) and superoxide dismutase (SOD) which is seen in the *S. nigrum* treated group G4 i.e. 74.430 ± 3.03 U/mg and 7.230 ± 0.27 U/mg respectively. Lowest value of CAT and SOD was seen in G2 i.e. 64.140 ± 1.67 U/mg and 4.87 ± 0.15 U/mg respectively. Administration of these antioxidants from *S. nigrum* fruit protects the body from lipid peroxidation. Li et al. (2021b) reported that intake of *S. nigrum* increase the antioxidative enzymes in rabbits. Huang et al. (2021) also studied the effect of ethanol extract of *S. nigrum* in phenylhydrazine induced oxidative stress.

Values are mean ± SD significant difference between Control and *S. nigrum*- treated Dawley rats by t-test; *P < 0.01.

Unit: Catalase (U/mg), Superoxide dismutase (U/mg). G1: control group which remained untreated G2: phenylhydrazine administrated rats G3: phenylhydrazine administrated rats with addition to standard drug G4: phenylhydrazine administrated rats with *S. nigrum* (1.0 g/kg b.w.) Different superscripts letters in the graph differ significantly (P < 0.01).

3.5 Effect of S. nigrum on phenylhydrazine induced hepatoxicity histological changes

Photomicrographs of the liver showing all the alternations in the rat's liver are presented in Figure 4. G1 photomicrograph indicated normal liver physiology. G2 photomicrograph showed altered hepatic structure due to phenylhydrazine (65 mg/kg b.w.

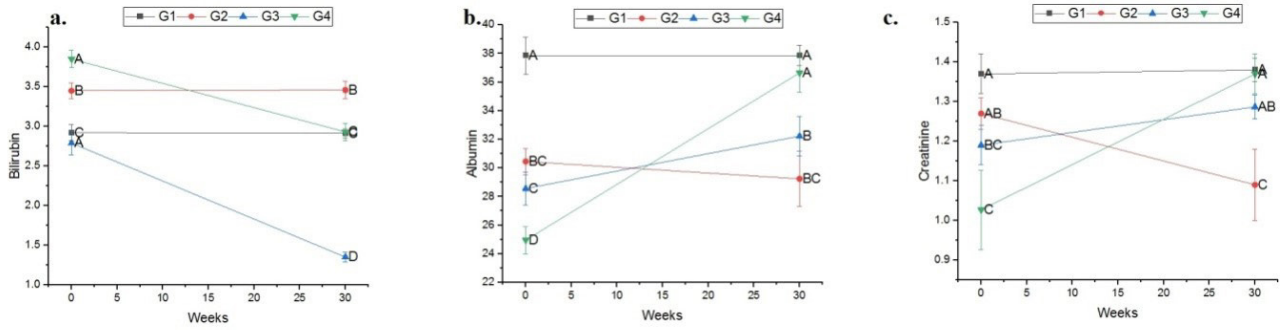

Figure 2. Effect of *S. nigrum* fruit on bilirubin (a), albumin (b) & creatinine (c).

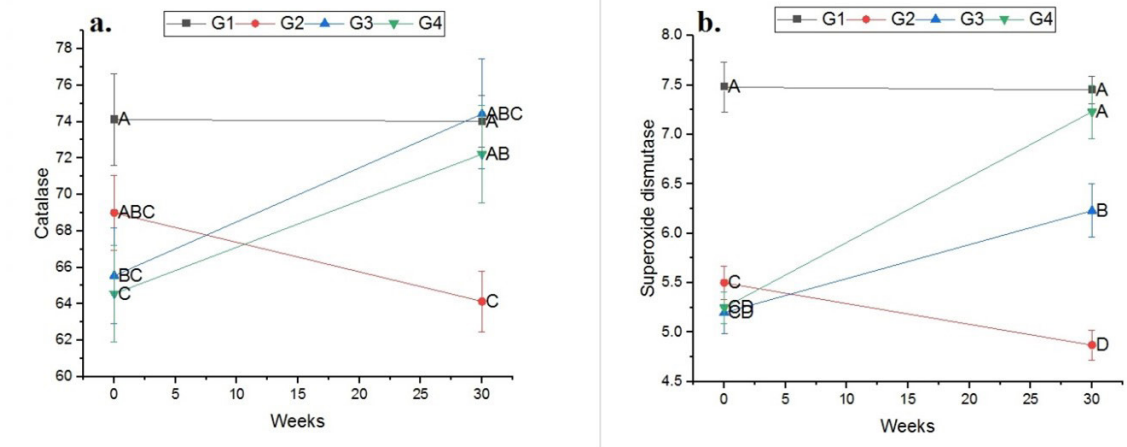

Figure 3. Effect of *S. nigrum* fruit on catalase (a) and superoxide dismutase (b).

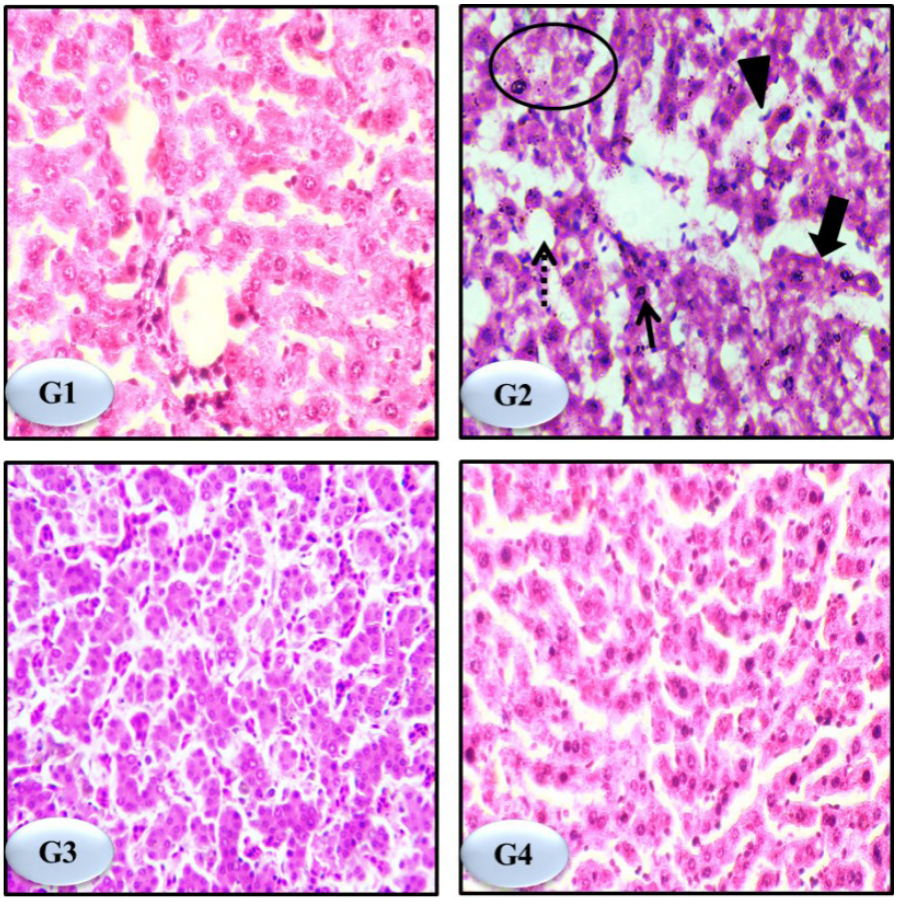

Figure 4. Histopathological indications of liver tissues.

for 8 days) which showed vacuolation, pyknotic nuclei, necrosis, congestion and atrophy of hepatocytes. G3 photomicrograph showed the medium to mild fatty degeneration. Normal histo-architecture in S. *nigrum* co-treated with Phenylhydrazine (G4) was seen in this group. Proper suppression of ballooning degeneration of liver cells can be seen in this group. All the scattered areas of necrosis were diminished.

Histopathological indications of liver tissues (H&E 10×). Normal Hepatocytes are observed in negative control (G1). Phenylhydrazine (65 mg/kg b.w. for 8 days) is Positive control (G2) Dotted Arrow (Vacuolation); Black Arrow (pyknotic nuclei); Necrosis(Oval); Thick Arrow (Congestion); Arrow head (atrophy of hepatocytes) Normal histo-architecture in silymarin and *S. nigrum* fruit co-treated with phenylhydrazine (G3 & G4).

4 Conclusion

S. nigrum fruit showed the protective effect due to the antioxidant potential. HPLC revealed the presence of many phenolic compounds which act as bioactive compounds against phenylhydrazine induced liver toxicity. *S. nigrum* fruit has significantly reduced the liver enzymes (ALT, AST and AP) and oxidative stress. Oxidative stress during liver toxicity is due to the increase production of reactive oxygen species (ROS) along with surge of superoxide anions. All the biomarkers linked with phenylhydrazine induced liver toxicity were reduced by the administration of *S. nigrum* fruit. Oxidative enzymes (SOD and CAT) levels were significantly increased with the intake of *S. nigrum* fruit in rats. Furthermore, normal hepatocyte structure was restored in Sprague Dawley rats treated with *S. nigrum* fruit. It is concluded from this research that *S. nigrum* fruit has plenty of active ingredients which serve as protection from free radical species. *S. nigrum* fruit serve as a therapeutic potential to treat acute liver toxicity by modulating oxidative stress through physiological pathways. Hence, *S. nigrum* fruit intake should be encouraged to ameliorate the acute liver toxicity.

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