

The antioxidant and hepatoprotective potential of *Solanum nigrum* against oxidative stress

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Abstract

The present study aimed to evaluate the phytochemical screening and in vivo antioxidant potential of *Solanum nigrum* against phenylhydrazine induced liver injury. The *Solanum nigrum* extract has significant in vitro antioxidant activity. Moreover, HPLC analysis of ethanolic *Solanum nigrum* extract showed revealed the presence of phenolic compounds. The Sprague Dawley rats were divided into four groups for in vivo analysis: G1 (negative control group), G2 (positive control group), G3 (rats receiving standard drug), and G4 (rats receiving *Solanum nigrum* extract i.e.1.0g/kg b.w.). The *Solanum nigrum* also reduced the level of liver enzyme, and bilirubin. An opposite trend was seen in the case of albumin, catalase, and superoxide dismutase. Histology slides also showed the normal cell structure of hepatocytes. Gene expression analysis demonstrates the upregulation of antioxidant and anti-apoptosis genes. Conclusively, *Solanum nigrum* has the ability to alleviate liver toxicity in an animal model by reducing oxidative stress and downregulating the apoptosis genes.

Introduction

S. nigrum is an 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 [1]. 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 [2]. *S. nigrum* consist of many constitutes like anthocyanins, anthocyanidins, flavonoids, tanins, vitamin C, vitamin E and small quantities of iron, zinc and selenium. Anthocyanins are antioxidants in nature and have ability to arrest the oxidative stress produced during Acute Liver Toxicity [3]. Being antioxidants in nature, they donate the pair of electrons and stabilize the free radicals in body. *S. nigrum* is one of the richest sources of anthocyanins after berries [4].

S. nigrum is one of the richest sources of phenolic compounds like gallic acid, quercetin etc. 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 [5]. The role of *S. nigrum* is significant against acute liver toxicity due to presence of active ingredients [6]. Out of all the parts, leaves and fleshy portion of *S. nigrum* are used mostly for therapeutic functions [7]. *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 [8]. About 2 million deaths per year occurs due to liver toxicity worldwide and 60% of them have acute toxicity [9].

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 [10]. All the liver enzymes and bilirubin values are increased with the intake administration of single dose of phenylhydrazine, which in return cases the lipid peroxidation [11]. In light of the aforementioned facts, the purpose of the study is to evaluate the potential of *S. nigrum* in

phenylhydrazine induced acute liver toxicity by using different parameters like aspartate aminotransferase (AST) and alanine transaminase (ALT) etc.

Materials And Methods

This section is provided as supplementary material.

Results And Discussion

Quantification of polyphenols

According to Peng *et al* [12], good antioxidant activities exhibited by plants extracts are due to the presence of poly-phenolic compound. Phenolic compounds present in *S. nigrum* are gallic acid, benzoic acid, quercitrin, ferulic acid and caffeic acid. The quantity of these phenolic compounds is shown in Table 1. [13] reported that the main phenolic compounds found in the extract of *S. nigrum* were gallic acid, p-coumaric and caffeic acid. It also includes rutin, gossypin and, epicatechin. [12, 14] conducted a study for the identification of phenolic compounds in reducing weight and body fat. In Huang *et al* [15] the phenolic extract was used to treat and prevent hepatocarcinoma. According to this study, this herb extract includes protocatechuic acid, gallo catechin and caffeic acid with the recovery time of 4.55%, 1.37% and 7.17%.

Table 1	
Phenolic compounds present in <i>S. nigrum</i>	
Phenolic Compounds	Concentration*
Gallic acid	6.02
Benzoic acid	0.37
Quercetin	1.23
Ferulic acid	0.18
Caffeic acid	0.08
*(µg/mg)	

Effect of *S. nigrum* on aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (AP)

Antioxidant potential of *S. nigrum* was checked by estimating the fluctuations in liver enzymes i.e. aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (AP). Statistical data showed that all the treatments have significant affected on the AST, ALT & AP (Figure1a-c). The level of all the liver enzymes were increased in phenylhydrazine treated rats. Lowest AST level was noted in the

G4 i.e. 215.75 ± 7.74 IU/L where *S. nigrum* intervention was given to rats (Figure1a). AST values in the G3 were higher as compared to G4. ALT is a chief indicator of hepatic injury and fluctuations occurs in the case of hepatotoxicity. ALT values were significant higher in G2 i.e. 108.77 ± 1.89 IU/L as compared to G4 i.e. 92.13 ± 3.41 IU/L (Figure1b). G3 showed high value i.e. 97.70 ± 4.03 IU/L as comparison to G4. 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 (Figure1c). Results from the study of [16] 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) [17]. The alterations in liver enzymes (ALT, AST and AP) upon administration of phenylhydrazine was also assessed by [18].

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

Bilirubin, albumin & creatinine were assessed to evaluate the potential of *S. nigrum* in Sprague dawley rats (Fig. 2). Highest value of bilirubin was present in G2 i.e. 3.4600 ± 0.11 μ mol/L (Fig. 2a). G4 showed decreased bilirubin level (2.93 ± 0.1 μ mol/L, $P < 0.01$) as compared to the positive control. G3 also showed the high values of bilirubin (2.9700 ± 0.06 μ mol/L) when compared to G4. This result also corresponds with the study of [19] 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 creatinine showed the values of 36.650 ± 1.36 g/L and 1.38 ± 1.08 μ mol/L respectively in G4 which is higher as compared to G2 (Fig. 2b&c). G3 showed the low values of albumin (32.220 ± 1.39 g/L) and creatinine (1.28 ± 1.26 μ mol/L) as compared to G4. This results also corresponds to the values of [20] where hydro alcoholic extract of *S. nigrum* was given to glycol induced toxicity. Veerapagu *et al* [21] 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.

Effect of *S. nigrum* on catalase (CAT) and superoxide dismutase (SOD)

Figure 3 revealed that the highest value of catalase (CAT) and superoxide dismutase (SOD) 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 as compared to G3. Lowest value of CAT and SOD is seen in G2 i.e. 64.140 ± 1.67 U/mg and 4.87 ± 0.15 U/mg respectively. Administration of these antioxidants protects the body from lipid peroxidation. Li *et al* [22] reported that intake of *S. nigrum* increase the antioxidative enzymes in rabbits. Haung *et al* [23] studied the effect of methanol extract of *S. nigrum* in phenylhydrazine induced oxidative stress.

Effect of *S. nigrum* on phenylhydrazine induced hepatotoxicity histological changes

Photomicrographs of the liver showing all the alternations in the rat's liver are presented in Fig. 4. Figure 4a was showing a G1 photomicrograph, which indicated normal liver physiology. Hepatocytes were arranged in hepatic cords. Nucleolus and chromatin material were also normal. Moreover, it was indicated that sinusoidal spaces were also ordinary. Figure 4b was showing a G2 photomicrograph with altered

hepatic structure due to phenylhydrazine. Fats start accumulating in different parts of the liver. All sinusoidal spaces were diminished and nucleus got condensed. Mild to severe inflammation occurred in the lobular region. Figure 4c is showing G3 in which a standard drug was given to rats to treat phenylhydrazine-induced liver toxicity. Less inflammation was seen in this case. Necrotic damage was also reduced due to standard drug administration. Medium to mild fatty degeneration can also be seen in this group. Figure 4d was showing the G4 group in which rats were fed with *S. nigrum* extract. Proper suppression of ballooning degeneration of liver cells can be seen in this group. All the scattered areas of necrosis were diminished. Nucleolus and chromatin material was also normal with only a few areas of congestion. There was a restoration of the normal structure of liver cells in the treatment group.

Gene Expression

Expression of Nrf2-ARE and BCL2 gene alongside BAX were analyzed to check the effectiveness of *S. nigrum* as shown in Fig. 5. Figure 5a showed that expression of Nrf2 was upregulated in G4 as compared to G2. Conversely, in other group G3, the expression was downregulated.. Previous studies [24] also support the activation of Nrf2-ARE in liver tissues, when oxidative stress was induced using different drugs. In another study [25], the pathway of Nrf2-ARE was observed in the early stage of hepatocarcinoma where upregulation of Nrf2 was seen when the treatment group was fed with phytochemicals. Similarly, BAX gene expression has increased in G4 as compared to other groups like G2 and G4. BCL2 gene is downregulated in G4 and upregulation has been in G2 and G3. Previous studies [26]; [27] also showed the same results where BAX induced the process of apoptosis in liver tissues in the early stages of cancer.

Conclusion

The *S. nigrum* showed the protective effect against phenylhydrazine induced liver toxicity. *S. nigrum* 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*. Oxidative enzymes (SOD and CAT) levels were significantly increased with the intake of *S. nigrum* in rats. Furthermore, downregulation of genes related to oxidative stress were observed in Sprague dawley rats treated with *S. nigrum*. It is concluded from this research that *S. nigrum* has plenty of phenolic compounds which act as an active ingredients. *S. nigrum* serve as a therapeutic potential to treat acute liver toxicity by modulating oxidative stress through genetic and other physiological pathways. Hence, *S. nigrum* intake should be encouraged to ameliorate the acute liver toxicity.

Declarations

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Availability of data and material

This article contains supplementary material, which is available to all users.

Author's contributions

Amna Alam wrote the main manuscript. Amna Sahar conceptualizes and supervises the whole research. Aysha Sameen assists in formatting and preparing of figures. Muhammad Naeem Faisal assists in all the experimental design.

Ethics declarations

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. 1,315), University of Agriculture Faisalabad, Pakistan.

Conflicts of Interests

The authors declare that they have no conflicts of interests.

Consent to participate

Not applicable

Consent to publication

All the authors give their consent to publish their research work in this journal.

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Figures

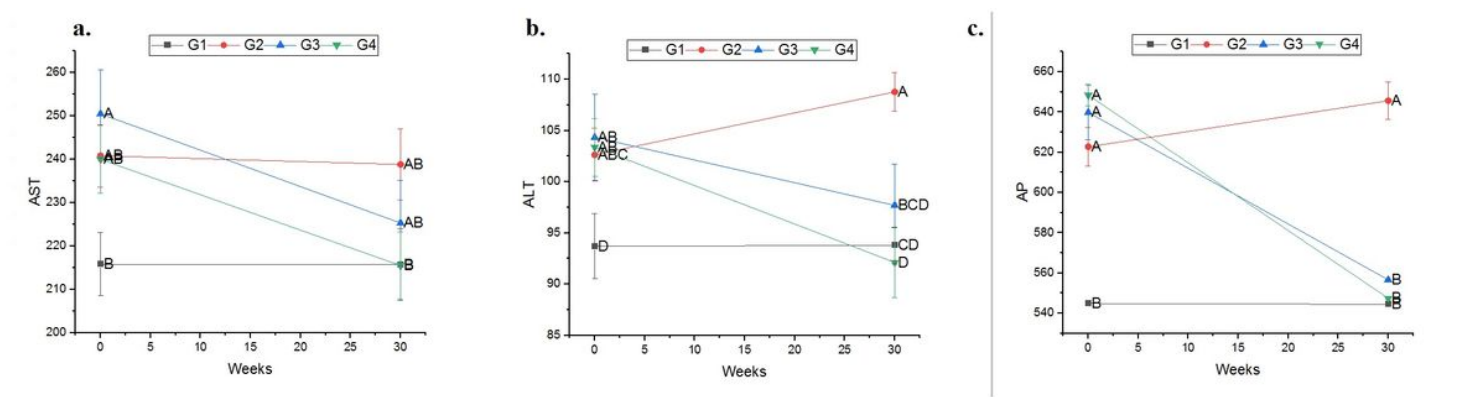


Figure 1

1a, b & c: Effect of *S. nigrum* on liver enzymes AST, ALT & AP

Values are mean $\pm$ SD significant difference between control and *S. nigrum*- treated dawley rats by t-test; *P<0.01 Unit: 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* (1.0 g/kg b.w.)

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

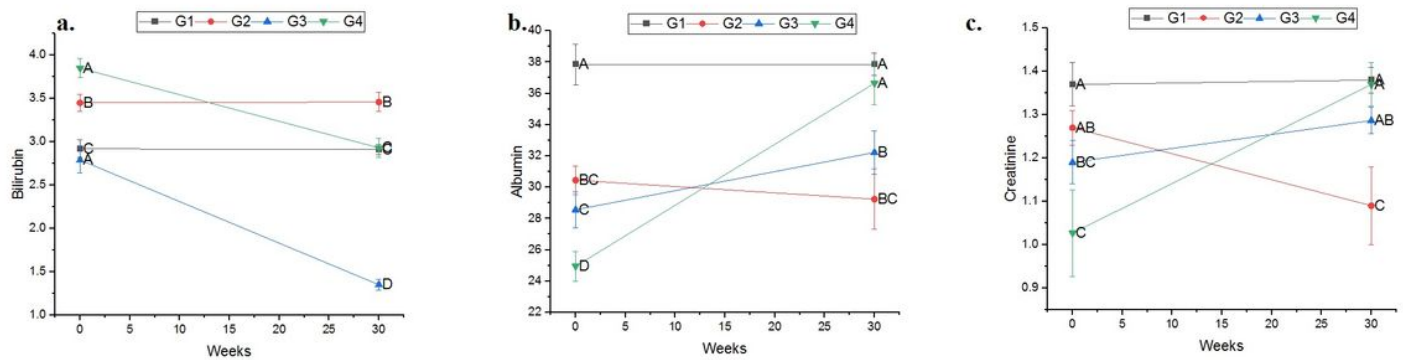


Figure 2

a, b & c: Effect of *S. nigrum* on Bilirubin, Albumin & Creatinine

Values are mean $\pm$ SD significant difference between Control and *S. nigrum*- treated Dawley rats by t-test,*P<0.01

Unit: Bilirubin ($\mu\text{mol/L}$), Albumin (g/L), Creatinine ($\mu\text{mol/L}$). 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* (1.0 g/kg b.w.)

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

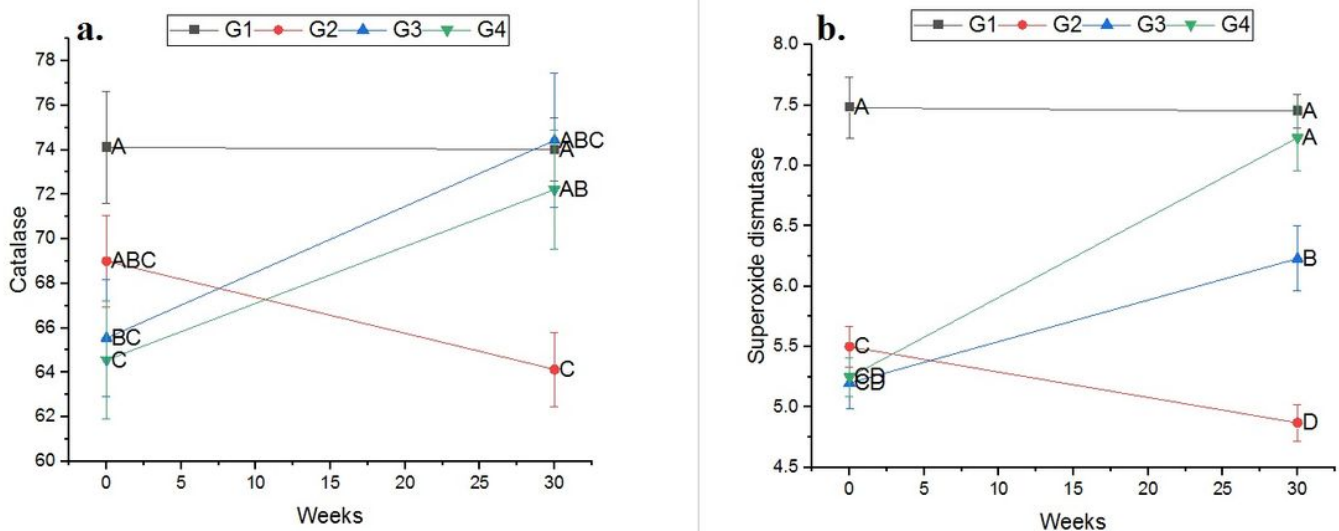


Figure 3

a & b: Effect of *S. nigrum* on Catalase and Superoxide dismutase

Values are mean $\pm$ 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 G 4: phenylhydrazine administrated rats with *S. nigrum* (1.0 g/kg b.w.)

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

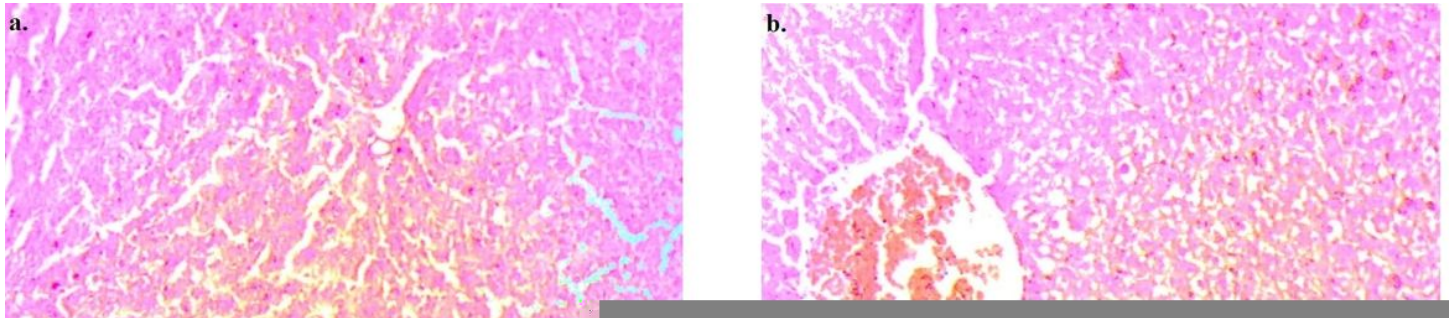


Figure 4

Histopathological indications of Liver

Histopathological indications of Liver tissues (H&E 10×).). a=G1: control group which remained untreated b=G2: phenylhydrazine administrated rats c=G3: phenylhydrazine administrated rats with addition to standard drug d=G 4: phenylhydrazine administrated rats with *S. nigrum* (1.0 g/kg b.w.)

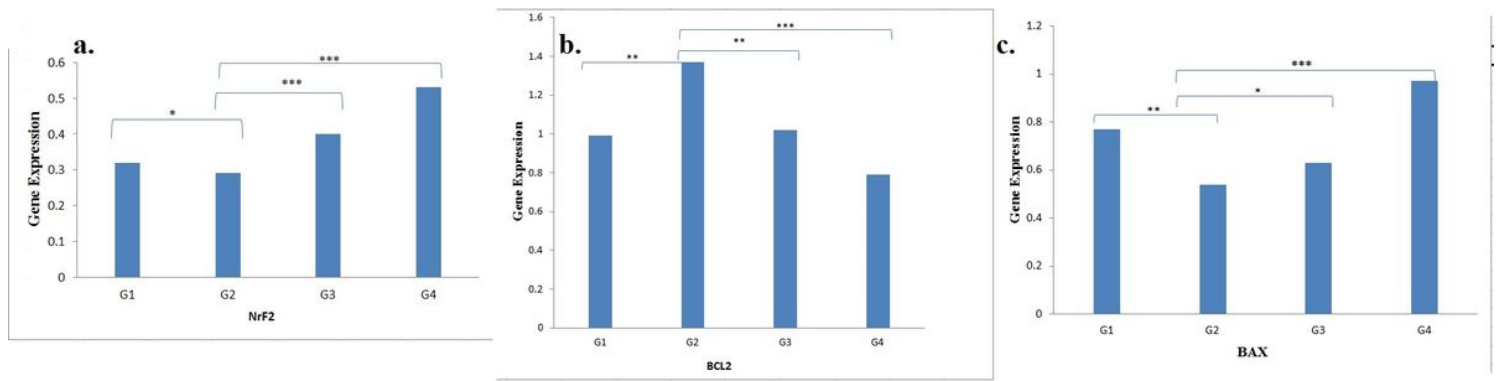


Figure 5

Gene expression analysis of antioxidant, apoptosis and anti-apoptosis gene markers

Significances are checked at $\leq 0.05 = *$; $\leq 0.01 = **$; $\leq 0.0001 = ***$

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* (1.0 g/kg b.w.)

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