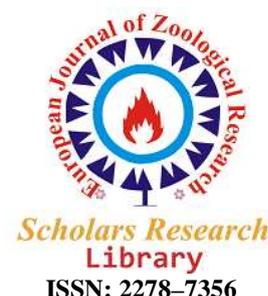




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European Journal of Zoological Research, 2013, 2 (2):19-25
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Hepatoprotective Activity of Aqueous-Ethanollic Extract of *Solanum nigrum* Against Nimesulide Intoxicated Albino rats

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ABSTRACT

Nimesulide, a 4-nitro-2-phenoxy methanesulphonamide is a very effective non-steroidal anti-inflammatory Drug (NSAID), but at higher doses it leads to the undesirable side effects, such as hepatotoxicity. The present study was designed to evaluate the hepatoprotective activity of *Solanum nigrum* in acute and chronic liver damage induced by Nimesulide. The degree of protection was measured by estimating the biochemical parameters like alkaline phosphatase (ALP), serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and total bilirubin (TB) level. The hepatoprotective activity of aqueous-ethanollic (30:70 %) extract at the doses of 100 mg/kg, 200 mg/kg and 300 mg/kg body weight, p.o., was compared with Silymarin (25 mg/kg, p.o.) treated animals. In acute liver damage, aqueous ethanollic extract of aerial parts of *Solanum nigrum* extract (Sn.E) in dose of 200 and 300mg/kg, p.o. significantly ($p < 0.001$) reduced the serum level of ALP, SGOT, SGPT and TB level. In the chronic liver damage, the higher doses (200 and 300 mg/kg, p.o.) of Sn.E were found to be more effective than the lower dose (100 mg/kg, p.o.). Furthermore, histopathological examination on the rat liver tissues supported the hepatoprotection. It is concluded that the aqueous ethanollic extract of aerial parts of *Solanum nigrum* possesses excellent hepatoprotective activity.

Key words: Nimesulide, Silymarin, *Solanum nigrum*, hepatoprotection.

INTRODUCTION

Liver is one of the most important and massive visceral organ present into substantial portion of abdomen. It is also called haper and made up of hepatocytes which carry out multiple metabolic processes essential for life. It is also involved in the removal of toxic materials from blood to avoid life threatening toxicities. Liver diseases have become one of the major causes of morbidity and mortality all over world. Many drugs and chemicals cause different types of hepatotoxicities that are highly variable, ranging from asymptomatic elevation of liver enzymes to fulminant hepatic failure. Throughout the world, the researchers have been in continuous search for some effective therapy for restoring the liver functions. The plant kingdom is undoubtedly one of the precious sources of new medicinal agents. Many plants and herbs play a major role in the management of various liver disorders. Different plant species in Pakistan like *Cichorium intybus*, *Alhagi maurorum* and *Rubia cordifolia* have been found to be hepatoprotective. Plant derived natural products such as terpenoids, alkaloids, flavonoids and steroids have gained significant considerations in recent years due to their various pharmacological properties including antioxidant and hepatoprotective activity.

Among the medicinal plants, *Solanum nigrum* (Family – Solanaceae) (known as black night shade, *Mako*) is a useful Indian medicinal plant which has been attributed with therapeutic properties to treat several diseases. It contains a number of chemical constituents, which are reported to possess antinociceptive, anti-inflammatory, antipyretic, cytotoxic and neoplastic effect [1], inhibitory effect on angiogenesis [2], antioxidant and neuropharmacological activities. Plant extract has reported hepatoprotective activity against CCl₄-induced chronic hepatic damages in albino rats. It significantly reduced the hepatotoxicity by lowering the level of certain hepatic enzymes [3]. The plant has been reported to contain two new pregnane saponins, solanigroside A1 and solanigroside B2, Degalactotigonin (I), and six new steroidal saponins, solanigrosides C-H (2-7), (+)-pinoresinol (I), (+)-syringaresinol (II), (+)-medioresinol (III), scopoletin (IV), tetracosanoic acid (V) and beta-sitosterol (VI) are found in whole plant [4]. The objective of the present study was designed to evaluate the possible hepatoprotective role of aqueous-ethanolic extract of *Solanum nigrum* against Nimisulide induced liver damage in rats.

MATERIALS AND METHODS

The approval of this study (Ref. No. 1560/Pharm) was taken from the Board of the Advanced Study and Research (BASAR), the Islamia University, Bahawalpur and the Institutional Ethical Committee, Faculty of Pharmacy and Alternative Medicine, the Islamia University, Bahawalpur.

2.1. Plant Material

Aerial parts of plant were collected from local fields of Sahiwal division, Punjab-Pakistan and identified by the botanist. The specimen was preserved in the herbarium vide Voucher No.SN-AP-04-12-044 at the Faculty of Pharmacy and Alternative medicine, the Islamia University of Bahawalpur, Pakistan.

2.2. Preparation of Extract

After washing, the Plant material was dried under shade. Dried material was ground to coarse powder by using electric grinder (National, Japan). About 710 g of ground powder was soaked in 3 L of 70% aqueous ethanol for five days with proper stirring. At the end of 5th day of maceration, it was filtered through muslin cloth and then through Whatmann filter paper No.1. Residue was again macerated to obtain more filtrate. This was repeated thrice and filtrate obtained after three soakings was evaporated by using rotary evaporator at 30-40°C. At the end, thick, viscous, semisolid paste of dark brown color was obtained. The extract obtained was 140g and percentage yield calculated was 19.71 %. The extract was packed in air tight container and labeled as Sn.E. It was then put in the refrigerator for future use.

2.3. Pharmacological materials

Diagnostics kits (ALP, SGOT, SGPT and TB), Ethanol, Formalin, Xylene, Paraffin Wax, Eosin, Hematoxylin, Canada balsam and Nimesulide. All the chemicals of analytical grade were purchased from Merck, Human-Germany and Nimesulide was donated by Sami Pharmaceuticals, Pakistan upon request. Silymarin was purchased from Abbott Laboratories, Pakistan. Ketamine and Diazepam were purchased from local Pharmacy.

2.4. Phytochemical Aanalysis

Different secondary metabolites are present in plant materials which exhibit various pharmacological activities [5]. Crude extracts were subjected to phytochemical analysis for identification of alkaloids, cardiac glycosides, steroids, tannins, and saponins. Following methods were used for analysis.

Tests for Saponins

Foam test: 500 mg of crude extract was dissolved in boiling water in test tube. Then it was cooled down and vigorously shaken to produce the forth [6]. Presence of forth indicated the saponins.

Tests for Tannins

Ferric chloride test: Extract was dissolved in 10 ml of distilled water and then filtered. 1% aqueous or alcoholic FeCl₃ was added in filtrate which produced intense green, purple, blue or black colour which indicated the tannins.

Iodine test: Extract was treated with dilute iodine solution. Formation of transient red colour indicated the presence of tannins.

Nitric acid test: extract was treated with dilute nitric acid and the formation of reddish to yellow colour indicated the presence of tannins.

Gelatin test: 0.5 g of extract was mixed with 1% gelatin solution containing 10% NaCl. Formation of white precipitates indicated the tannins [7].

Test for Alkaloids

500-600 mg of crude extract was treated with 8 ml of 1% HCl, warmed on water bath and then filtered and divided in to four test tubes.

Hager's test: 2 ml of filtrate was mixed with few drops of Hager's reagent (saturated aqueous solution of picric acid). Appearance of turbidity or yellow precipitates indicated the presence of alkaloids.

Wagner's test: 2 ml of filtrate was mixed with few drops of Wagner's reagent. Appearance of reddish brown precipitates indicated the presence of alkaloids.

Dragendroff's test: 2 ml of filtrate was mixed with Dragendroff's reagent. Appearance of turbidity or precipitates indicated the presence of alkaloids.

Mayer's test: 2 ml of filtrate was mixed with Mayer's reagent. Appearance of turbidity or precipitates indicated the presence of alkaloids [8].

Tests for Glycosides

Keller-Kiliani Test: Took extract solution in test tube and added few drops of FeCl_3 in it. Concentrated CH_3COOH and concentrated H_2SO_4 were added carefully along the wall of test tube. Reddish brown coloration at the junction of both layers and bluish green color at the upper layer indicated the presence of glycosides.

Tests for terpenes and Sterols

Liebermann-Burchard Test: 30 ml of crude extract was added in petroleum ether. Petroleum ether was evaporated to get dry residue. Residue was then extracted with 20 ml of chloroform and the chloroform layer was then treated with anhydrous sodium sulphate. 0.5 ml of acetic anhydride was mixed with 5 ml of chloroform layer. Then two drops of concentrated H_2SO_4 was added which gave green, blue and pink to purple colours. Green to pink color indicated the presence of sterols while pink to purple colours are proof of presence of triterpenes [9].

2.5. Experimental animals

Sprague-Dawley albino rats of both sexes weighing 180-200 g were used in this study. All animals were kept in Polycarbonated cages of size $47 \times 34 \times 18 \text{ cm}^3$ in animal house of Faculty of Pharmacy and Alternative Medicine. They were provided standard temperature ($25 \pm 2^\circ\text{C}$) and humidity (50-55 %) along with exposure of 12:12 hours light and dark cycle till end of study. Before initiation of experiments, the rats were acclimatized for one week and provided with free excess of water and food.

2.6. Induction of Hepatotoxicity

Nimesulide was used to induce hepatotoxicity in albino rats. Nimesulide was solubilized in Dimethyl sulfoxide (DMSO) and administered orally on daily basis.

2.4. Hepatoprotective activity of crude extract of *Solanum nigrum*

For evaluation of hepatoprotective activity of crude extract of *Solanum nigrum*, animals were divided into seven groups with seven animals in each group. Group-I received normal saline at dose of 5ml/kg p.o. once daily. Group-II was given DMSO at dose of 5ml/kg p.o. Group-III received Nimesulide 100 mg/kg p.o. for seven days to produce hepatotoxicity. Group IV was Standard Control given Silymarin alone for first eight days at dose of 25 mg/kg p.o. and then along with Nimesulide (100 mg/kg p.o.) for further seven days. Group V-VII were given crude extract alone at dose of 100, 200 and 300 mg/kg p.o., respectively for first eight days and then Nimesulide in dose of 100 mg/kg p.o. along with plant extracts to study the hepatotoxicity for further seven days.

At the end of fifteen days of treatment, all the animals were scarified by using Ketamine as an anesthetic agent in a dose of 50 mg/kg i.p. Animals were dissected to expose heart. Blood was withdrawn in sterile disposable syringe directly from the heart of rat by cardiac punctures. Then it was subjected to centrifugation at 2500 revolution per minute (rpm) for 15 minutes. Serum was used for investigation of hepatic enzymes. The livers were harvested, washed in the normal saline, blotted the filter paper and weighed and subsequently subjected to histopathological examination.

2.7. Statistical analysis

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by student t test. The values were expressed as mean \pm SE and $P \leq 0.001$ was considered significant.

RESULTS

3.1. Phytochemical investigations

The preliminary phytochemical investigation of crude extract of *Solanum nigrum* showed that it highly contains saponins, along with tannins, cardiac glycosides, terpenes and sterols (Table 1).

Table: 1. Phytochemical constituents of *Solanum nigrum* (Sn.E)

Sr. #	Phytochemical Tests	Phytochemical Constituents
Saponins		
1	Foam Test	+ve
2	Haemolysis Test	+ve
Tannins		
1	Iodine Test	-ve
2	Ferric Chloride Test	+ve
3	Nitric Acid Test	+ve
4	Gelatin Test	+ve
Alkaloides		
1	Hager's Test	+ve
2	Wagner's Test	+ve
3	Mayer's Test	-ve
4	Dragendorff Test	-ve
Cardiac glycosides		
1	Keller Killani test	+ve
Terpenes and sterols		
1	Liebermann-Burchard test	+ve

Note: (+) and (-) signs report the relative presence and absence of constituents in Sn.E.

3.2. Biochemical parameters

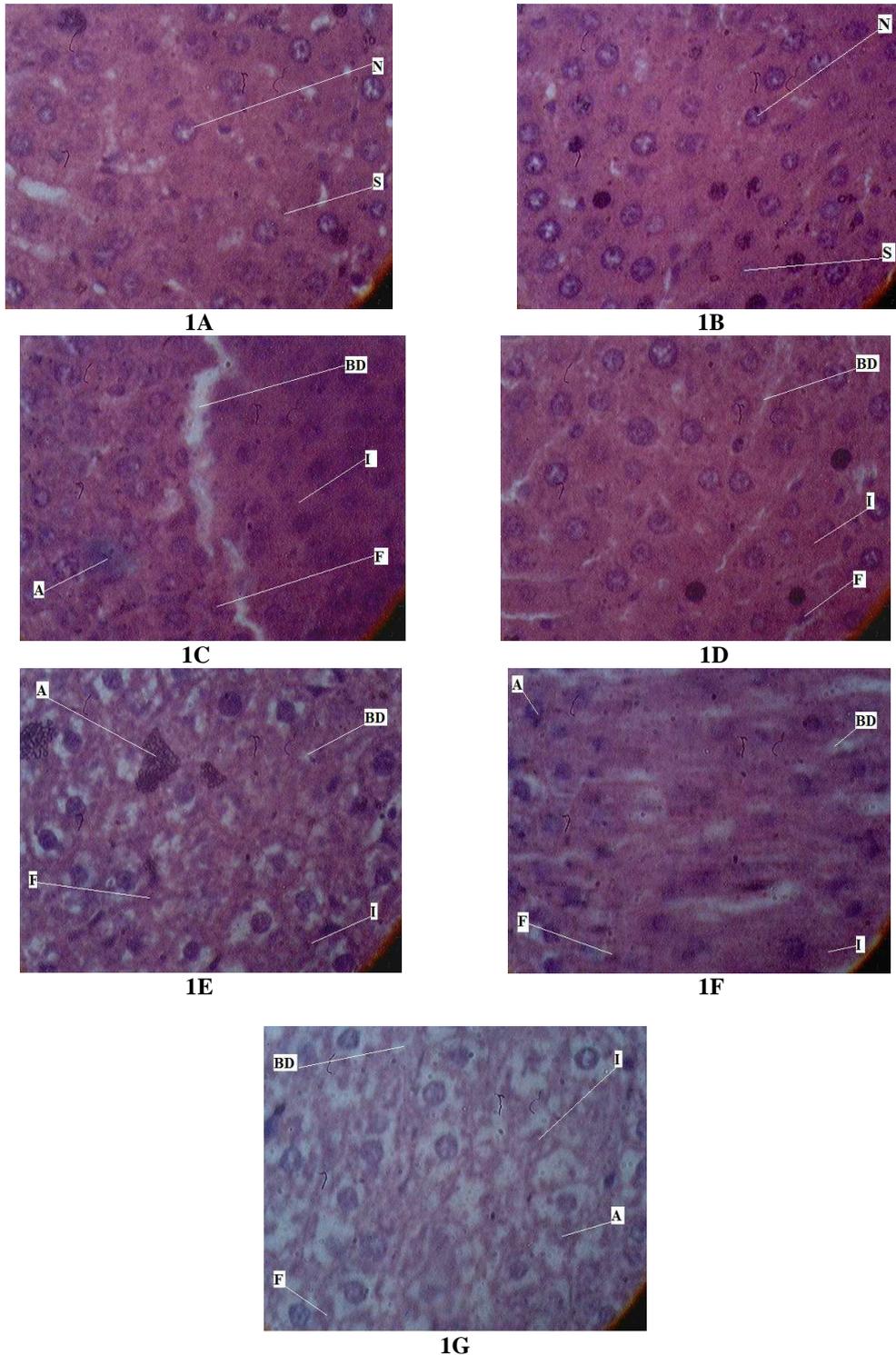
Experimental studies indicated that the values of serum ALP, SGOT, SGPT and TB were quite close in normal control and vehicle control groups. This indicated that DMSO had least effects on serum enzymes level in albino rats. The levels of serum marker enzymes in intoxicated group were very high in comparison to normal control group. It represented that Nimesulide clearly produced hepatotoxicity in rats. However, there was remarkable reduction in serum enzymes in standard control group. Sn.E in dose of 100 and 200 mg/kg reduced the level of enzymes almost equally but Sn.E 300 mg/kg reduced the enzymes level more effectively.

Table: 2. Effects of different doses of *Solanum nigrum* extract (Sn.E) on ALP, SGOT, SGPT & TB level in Nimesulide intoxicated albino rats.

[Values are mean \pm SE from 7 animals in each group]

Sr. #	Treatment Group	Level of ALP (IU/L)	Level of SGOT (IU/L)	Level of SGPT (IU/L)	Level of TB (IU/L)
1	Normal Control	220.77 \pm 15.56	112.24 \pm 5.27	51.60 \pm 4.35	0.85 \pm 0.07
2	Vehicle Control	219.17 \pm 15.82	108.81 \pm 4.22	51.04 \pm 4.35	0.86 \pm 0.08
3	Intoxicated Control	889.01 \pm 24.71 ^{###}	223.29 \pm 7.57 ^{###}	115.57 \pm 5.67 ^{###}	3.60 \pm 0.16 ^{###}
4	Standard Control	260.16 \pm 17.81 ^{***}	116.69 \pm 5.76 ^{***}	58.03 \pm 3.34 ^{***}	0.95 \pm 0.15 ^{***}
5	Sn.E 100 mg/kg	553.84 \pm 32.67 ^{***}	203.10 \pm 6.95 ^{ns}	93.61 \pm 2.36 ^{**}	3.14 \pm 0.10 ^{ns}
6	Sn.E 200 mg/kg	566.84 \pm 31.16 ^{***}	190.49 \pm 11.35 [*]	84.19 \pm 5.19 ^{***}	2.75 \pm 0.09 ^{***}
7	Sn.E 300 mg/kg	428.40 \pm 19.94 ^{***}	165.13 \pm 9.12 ^{***}	77.14 \pm 3.61 ^{***}	2.61 \pm 0.14 ^{***}

P-values: ### ≤ 0.001 vs. vehicle control, ^{ns} > 0.05 , ^{} < 0.05 , ^{**} < 0.01 , ^{***} < 0.001 vs. intoxicated control*



(N= Nucleus, S= Sinusoid, BD= Ballooning-degeneration, F=Fibrosis, I= Inflammation and A= Apoptosis)

Fig: 1. Photomicrographs (100X) of liver tissues of different groups of albino rats.

(1A) Normal control; (1B) Vehicle control; (1C) Intoxicated control; (1D) Standard control; (1E) Sn.E 100 mg/kg; (1F) Sn.E 200 mg/kg; (1G) Sn.E 300 mg/kg.

3.3. Histopathological examination

Histopathological examination of the liver tissue from Nimesulide treated animals revealed that it had produced profound ballooning degeneration, inflammation, apoptotic cells, fibrosis and congestion especially in sinusoids. Pretreatment with Silymarin, Sn.E (100 mg/kg po), Sn.E (200 mg/kg po) and Sn.E (300 mg/kg po) reduced the inflammation and degenerative changes.

DISCUSSION

Some hepatotoxins and chemicals decrease the level of hepatic DNA, RNA and protein contents. Substances having potential to increase the production of DNA, RNA and proteins, prevent the hepatotoxicity [10]. Certain hepatotoxic metabolites like thioacetamide S-oxide produce centrilobular necrosis. It produces changes in nucleolous and stimulates more and more production of guanine and cytosine with concomitant decrease in ribosomal RNA in cytoplasm [11].

Nimesulide raised the level of various liver enzymes like ALP, SGOT and SGPT etc. Histopathological studies indicated that it produced severe inflammation in hepatic cells along with fibrosis, apoptosis and ballooning-degeneration as shown in figure 1C. Proposed mechanism of toxicity is that nitroarene group of nimesulide is metabolized in reactive intermediate which causes oxidative stress, covalent binding and mitochondrial injury [12]. Hepatotoxic substances (CCl₄ and Paracetamol) produce histopathological changes (steatosis and fibrosis) in hepatocytes [13].

Hepatoprotective activity of Sn.E might be due to the presence of saponins, tannins, glycosides, terpenes and sterol present in plant. Tannins are well recognized due to their hepatoprotective action. Saponins like saikosaponins inhibit lipid peroxidation by scavenging reactive and toxic species [14]. Alkaloids, flavonoids and triterpenoids are phytochemical constituents of *Solanum* species. Steroidal alkaloids of these species have hepatoprotective role. *Solanum nigrum* prevented thioacetamide induced liver damage in mice by inhibiting hepatic hydroxyl proline and α -smooth muscle acting protein levels [15]. Saponins present in plant extract might have role in scavenging of free radicals produced in liver by metabolism of Nimesulide. Liver sections of randomly selected rats from all groups under light microscope represented the hepatocellular changes which occurred in different groups. Sn.E produced fewer score of ballooning-degeneration, apoptosis, inflammation and fibrosis as shown in figures 1A-1G.

CONCLUSION

On the bases of results obtained, it is concluded that Sn.E exhibited hepatoprotective activity in Nimesulide intoxicated albino rats. There was marked reduction in level of four liver markers ALP, SGOT, SGPT and TB by the use of extract in 300 mg/kg as compared to other two doses.

The present investigations strongly strengthens the use of *Solanum nigrum* as hepatoprotective plant because it was scientifically proved that the plant is a potential source of useful drug due to the presence of phytochemical constituents. So it can be used for the treatment of hepatic diseases and also exploited for the use in pharmaceutical industries. However, further studies are required to isolate the active principle form the crude extract for proper drug development.

Acknowledgement

The authors of this publication are thankful to the Faculty of Pharmacy and Alternative Medicine, the Islamia University of Bahawalpur, Pakistan for providing all the necessities used in this research.

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