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DEPARTMENT OF ANATOMY

The Effect of *Solanum nigrum* L. on Histopathology of Liver and Kidneys of Rats

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List of abbreviations

ALP: alkaline phosphatase

ALT: alanine transaminase

ARF: acute renal failure

AST: aspartate aminotransferase

CAT: catalase

CCl_4: carbon tetrachloride

EDTA: ethylenediaminetetraacetic acid

GGT: gamma glutamyl transferase

GPx: glutathione peroxidase

GSH: reduced glutathione

Hb: hemoglobin

Liv. 52: a mixture of seven herbs extract (*Achillea millefolium* L., *Capparis spinosa* L., *Cassia occidentalis* L., *Cichorium intybus* L., *Solanum nigrum* L., *Tamarix aphylla* L. and *Terminalia arjuna* R.) that are used for treatment of liver diseases.

MDA: malondialdehyde

PCV: packed cell volume

PVP: polyvinylpyrrolidone

p.o: per mouth

ROS: reactive oxygen species.

SNFEt: *Solanum nigrum* fruits extract

SOD: superoxide dismutase

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Summary

Solanum nigrum L. is commonly called black nightshade that belongs to Solanaceae (potato) family. It is a fairly common herb or short-lived perennial shrub, found in many wooded areas, as well as disturbed habitats.

Various experimental based scientific studies were conducted in order to investigate the efficacy and safety of S. nigrum extract. It was shown that this plant has various bioactive ingredients such as alkaloids, solanins, saponins, flavonoids, tannins, steroidal glycoalkaloids, steroidal genin and vitamins. These constituents are responsible for diverse activities including anti-inflammatory, anti-bacterial, anti-diabetic, anti-fungal, anti-oxidant, hepatoprotective, nephroprotective and cytoprotective effects. In addition, these bioactive constituents have free radical scavenging capacity and anti-lipid peroxidation activities by stabilization of plasma membranes as well as repair of liver and kidney tissue damage.

Moreover, the result of this review showed that S. nigrum whole plant extract and S. nigrum fruits extract have the capacity to reverse serum levels of ALP, ALT, AST and bilirubin of liver and kidney damages to near normal levels if pathological change occurs. The S. nigrum whole plant extract and S. nigrum fruits extract were found to be safe for the liver and kidney parameters up to 5ml/kg doses. But this extract would have been toxic above 5ml/kg which is considered to be elevated dose. So that safe dosage needs to be identified for children and pregnant women because children have less body resistance and pregnant women may be susceptible to abort since it may induce uterine contraction.

Therefore, further studies are required to isolate the active ingredients from the extract of S. nigrum for proper drug development to treat the above mentioned health problems and to conduct further clinical trials.

1. Introduction

1.1. Traditional medicinal plants

More than 3.5 billion people in the developing world rely on traditional medicinal plants as components of their healthcare (Balick and Cox, 1996). This global utilization of medicinal plants has considerably increased in the last two decades. As elsewhere in Africa, local people in Ethiopia, by large employed plant based traditional medicine to treat ailments arising from worms, fungi, virus and protozoa (Abebe, 2001).

In Ethiopia, about 80% of the human population and 90% of the livestock rely on traditional medicine and the medicinal plants have shown very effective medicinal value for some ailments of humans and livestock. In addition, the acquisition and transfer of indigenous knowledge on traditional medicine, in most developing countries including Ethiopia, is passed from one generation to the next by word of mouth. Nowadays, more than ever, researchers of natural products-based drugs are going back to nature for the search of more effective and safe new drugs. About one thousand identified medicinal plant species are reported in the Ethiopian Flora. Although plant based natural medicines are popularly acclaimed to be safe, scientists advocate the proper toxicological studies in order to ensure safety in the use of herbal medicines (Endashaw, 2007).

According to Schiefer *et al*. (1997), toxicology encompasses the study of the adverse effects of chemical and physical agents on living organisms and groups of organisms. This deals with adverse effects ranging from acute to (chronic) long term i.e. acute toxicity testing is done by using single or repeated doses with observation for 14 days (typically tests for lethality or skin irritancy); subacute toxicity testing is done by using repeated doses for up to 90 days whereas chronic toxicity testing is done by using repeated doses for up to 2 years (including carcinogenicity testing).

Most species in the Solanaceae family are poisonous to humans as well as to livestock. The toxic effects of the plants are mainly reported in the older literature. For instance, deadly nightshade contains tropane alkaloids. The toxin, when ingested by humans in large quantities, causes anticholinergic effects. Although *S. nigrum* is considered to be an edible plant, its toxicity is mainly due to the presence of solanine, a glycoalkaloid causing varying degrees of toxicity in a dose-dependent manner. The symptoms of poisoning in humans due to solanine are reported to include nausea, vomiting, diarrhea, headache, dizziness, loss of speech, fever, sweating, tachycardia, pupil dilation, blindness, mental confusion, convulsions, coma, and death (Jain *et al*., 2011).

The amount of toxic compound in a plant depends on the climate, soil type, season, and maturity. The green unripe berries are generally considered more toxic than the ripe berries. It is probable that by boiling the plant, the toxic components are destroyed as the plant is reported to be edible after cooking. Traditionally, consumption of nightshade vegetables like tomato, potato, and eggplant has been considered to be problematic for arthritic patients. It has been reported that solanine present in the green parts of these vegetables is probably responsible for joint pain. Most toxic effects of drugs occur at a predictable time after administration, and such toxic effects caused by a drug are similar in human and some other animals. This fact serves as a principle for use of animal models in toxicological studies. Thus, continuous use and the growing demand for herbal therapies have restored the quest for validating the efficacy and safety or toxic implications of medicinal plants (Erasto *et al*., 2007; Jain *et al*., 2011).

1.2. *Solanum nigrum* L.

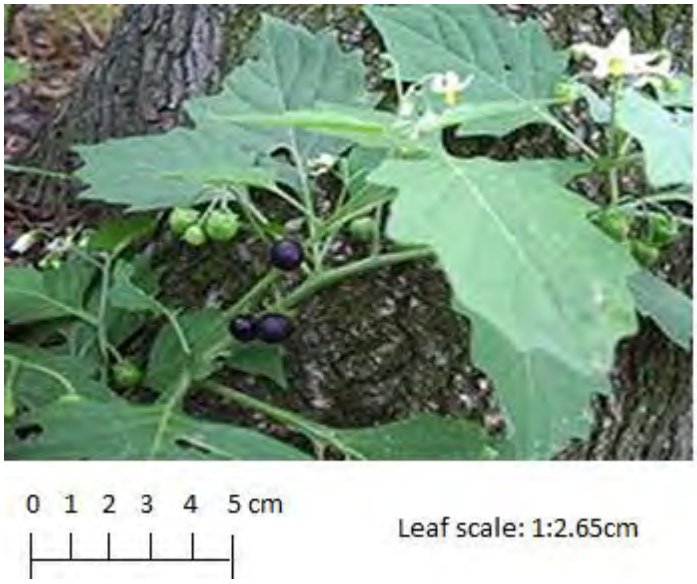
1.2.1. Taxonomy of *Solanum nigrum* L.

Solanum nigrum (*S. nigrum*) is a worldwide weed of arable land, gardens, rubbish tips, soils rich in nitrogen, in moderately light and warm situations which occur from sea to montane levels (Jennifer and James, 1997). It has vernacular name, black nightshade (English), Tikur-awitt (Amharic) and Muiulo (Afan Oromo). *S. nigrum* belongs to the kingdom Plantae (plants); subkingdom Tracheobionta (vascular plants); superdivision of Spermatophyta (seed plants); division of Magnoliophyta (flowering plants); class of Magnoliopsida (dicotyledons); subclass of Asteridae; order of Solanales; Family of Solanaceae (Potato family); genus of *Solanum* (nightshade) and species of *Solanum nigrum* L. (black nightshade) (Edmonds and Chewya, 2002).

1. *S. nigrum* L. subsp. *nigrum*: glabrous to slightly hairy with appressed non-glandular hairs.
2. *S. nigrum* L. subsp. *schultesii* (Opiz) Wessley: densely hairy with patent, glandular hairs.

1.2.2. Botanical description of *Solanum nigrum* L.

S. nigrum is a fairly common herb or short-lived perennial shrub, found in many wooded areas, as well as disturbed habitats. It is characterized by their lack of prickles and stellate hairs, their white flowers and their green or black fruits arranged in an umbelliform fashion. It has a height of 30 to 120 cm (12 to 48 in), leaves 4 to 7.5 cm (1.5 to 3 in) long and 2 to 5 cm (1 to 2.5 in) wide; ovate to heart-shaped, with wavy or large-toothed edges; both surfaces hairy or hairless; petiole 1 to 3 cm (0.5 to 1 in) long with a winged upper portion. The flowers have petals greenish to whitish, recurved when aged and surround prominent bright yellow anthers. The berry is mostly 6 to 8 mm (0.3 to 0.8 in) diameter, dull black or purple-black (Venkateswarlu and Krishna, 2007).



Solanum nigrum L.

Black berries of *Solanum nigrum* (unripe)

Red berries of *Solanum nigrum* (ripe)

Fig. 1. Leaves and berries of *S. nigrum* L.

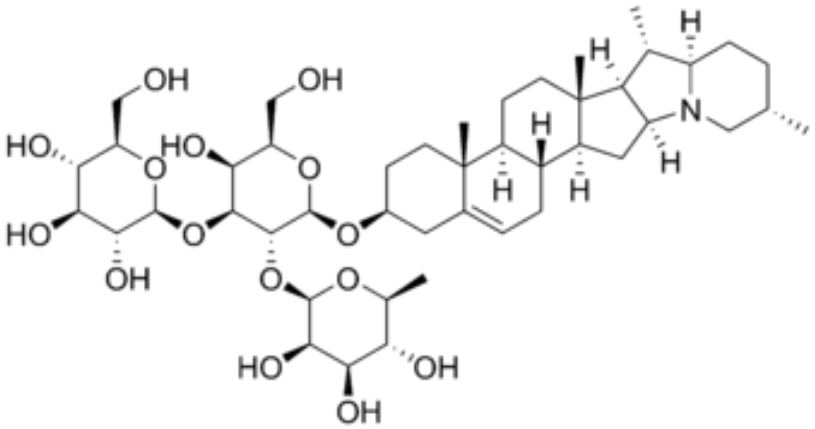
(Source: Edmonds and Chewya, 2002))

The toxicity of *S. nigrum* varies widely depending on the variety, and poisonous plant experts advise to avoid eating the berries unless they are a known edible strain (Turner and Aderka, 1997). Toxin levels may also be affected by the plant's growing conditions (Edmonds and Chewya, 2002).

1.3. Phytochemical property of *Solanum nigrum* L.

Phytochemicals are the secondary metabolites that have several subgroups, possessing various bioactivities such as antioxidant, antimicrobial, antivirus and anticancer. Even today, because of the belief that medicinal plants are safe and effective, most of the plant products are being used in local conventional systems of medicine. The unripe fruit of *S. nigrum* contain the highest concentration of toxin particularly solanine, a glycoalkaloid is found in most parts of this plant. The level of toxin in the berries is greatly reduced by ripening and when ripe, the berries are the least toxic and are sometimes eaten without ill effects. In addition, the ripe berries are eaten raw as fruits and are used in pies and preservative in many regions of the world (Aali *et al*., 2010; Din *et al*., 2012).

All parts of the plant can be poisonous, containing toxic glycoalkaloids at 0.524% (dry weight), including solamargine, solasonine and solanine. The toxins are most concentrated in the unripe green berries, but also occur in ripe berries. Solanine levels in *S. nigrum* can be extremely toxic and potentially fatal (Schep *et al*., 2009). Its toxicity is mainly due to the presence of active ingredient solanine, a glycoalkaloid causing varying degrees of toxicity in a dose-dependent manner (Jain *et al*., 2011).



Solanine Source**:** Edmonds and Chewya (2002)

According to Schep *et al*. (2009) poisoning symptoms are typically delayed for 6 to 12 hours after ingestion. The symptoms of poisoning in humans due to toxicity of solanine are fever, headache, sweating, nausea, vomiting, abdominal pain, diarrhea, drowsiness, dizziness, loss of speech, tachycardia, pupil dilation, blindness, mental confusion, convulsions, coma, and death. Death from ingesting plant parts results from cardiac arrhythmias and respiratory failure. Children have died after eating unripe berries, and consumption has caused livestock fatalities. Livestock have also been poisoned from nitrate toxicity by grazing the leaves of *S. nigrum*.

Although numerous texts state that the cooked ripe fruit of black nightshade is safe to eat, detoxification cannot be attributed to normal cooking temperatures because the decomposition temperature of solanine is much higher at about 243°C. There are ethnobotanical accounts of *S. nigrum* leaves and shoots being boiled as a vegetable with the cooking water being discarded and replaced several times to remove toxins (Tull, 1999; Edmonds and Chewya, 2002).

S. nigrum has been widely used as a food since early times, and the fruit was recorded as a famine food in 15^{th}C in China. Despite toxicity issues with some forms, the ripe berries and boiled leaves of edible strains are eaten. The thoroughly boiled leaves although strong and slightly bitter flavoured are used like spinach as horta, in fataya pies and quiches. The ripe black berries are described as sweet and salty, with hints of liquorice and melon (Irving, 2009).

In Ethiopia, the ripe berries are picked and eaten by children while during famines all affected people would eat berries. In addition the leaves are collected by women and children, who cook the leaves in salty water and consumed like any other vegetable. Farmers in the Konso Special Woreda ; *S. nigrum* is used as a food source until their crops are ready. The Welayta people in the nearby Wolayita Zone do not weed out *S. nigrum* that appear in their gardens since they likewise cook and eat the leaves (Zemede, 1995).

S. nigrum is an important ingredient in traditional Indian medicines. Infusions are used in dysentery, stomach complaints and fever. The leaves are known to be used to treat headache and diseases of nose, ringworm, heart liver and kidney ailments, wounds, burns and toothache. The ethnomedicinal information reveals that the juice of dried leaves of *S. nigrum* is used for lowering blood sugar level. The juice of the plant is used on ulcers and other skin diseases. The fruits are used as a tonic, laxative, appetite stimulant and also for treating asthma and "excessive thirst" (Kaushik *et al*., 2009). In North India, the boiled extracts of leaves and berries are used to alleviate liver-related ailments, including jaundice and the juice from its roots is used against asthma and whooping cough (Maharana *et al*., 2010).

S. nigrum is a widely used plant in oriental medicine where it is considered to be anti-tumorigenic, anti-oxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic. Chinese experiments confirm that the plant inhibits growth of cervical carcinoma in mice (Jian *et al*., 2011b; Senthilnath *et al*., 2013).

1.4. Objective of the project

1.4.1. General objective of the project

- To assess the effect of *S. nigrum* L. on histopathology of liver and kidneys of rats

1.4.2. Specific objective of the project

- To review the effect of *S. nigrum* L. on histopathology of liver of rats
- To review the effect of *S. nigrum* L. on histopathology of kidney of rats

2. Review of published papers on pharmacologic and toxic effects of *S. nigrum*

2.1. Pharmacologic effects of *S. nigrum* L.

Plants are essential in our environment. Some plants or plant parts are actually used for the treatment of human or animal diseases, in either pharmaceuticals or herbal remedies. The advice of an experienced herbalist should be sought before plants are used medicinally or for making herbal teas (Schiefer *et al*., 1997).

In order to explore the hepatoprotective activity of *S. nigrum* the whole dried plant and its fruit were separately powdered mechanically with herb grinder. The powder of whole plant and fruit were mixed manually and the prepared powder was kept in dry, clean, airtight glass jar and stored at 4°C until used. 100 g of the prepared powder was macerated in 500 ml of distilled water for 24 hours. It was then filtered through a 1mm mesh sieve and the filtrate was dried in Petri dishes and concentrated to a dark green residue by heating at 40°C, till complete evaporation of water was achieved (Kumar *et al*., 2013).

Thirty six albino rats were used in this study were divided into six experimental groups: Group 1 rats received normal saline 2ml/100g/day orally in addition to the standard rat pellet diet and tap water. Group 2 rats received 1 ml/kg of a 50% v/v solution of CCl_4 in olive oil intraperitoneally. Group 3 rats received Liv. 52 syrup (a mixture of extracts of seven herbs (*Achillea millefolium* L. (aerial parts), *Capparis spinosa* L. (root), *Cassia occidentalis* L. (seed), *Cichorium intybus* L. (seed), *Solanum nigrum* L. (whole plant), *Tamarix aphylla* L. (whole plant) and *Terminalia arjuna* R. (bark)) used for the treatment of various liver diseases) (2 ml/kg) orally for 20 days followed by CCl_4 intraperitoneally as in Group 2. The Liv. 52 were administered by gavage method with animals fasted 3-4 hours prior and 1 hour after administration to ensure proper absorption. CCl_4 dose was given concomitantly with the last (20^{th} day) dose of Liv. 52. Group 4 rats received the *S. nigrum* extract in the dose of 2ml/100g/day twice daily orally for the duration of 10 days followed by CCl_4 intraperitoneally as in Group 2 on the last day. Group 5 rats received *S. nigrum* extract (2ml/100g/day orally twice daily) for the duration of 20 days followed by CCl_4 intraperitoneally as in Group 2 on the last day. Group 6 rats received *S. nigrum* extract (2ml/100g/day orally twice daily) for the duration of 30 days followed by CCl_4 intraperitoneally as in Group 2 on the last day (Kumar *et al*., 2013).

ALT level in normal saline treated group was 25.87 ± 5.30 IU/l. It was found to be significantly increased ($p<0.001$) following administration of CCl_4 while pretreatment with known hepatoprotective preparation Liv. 52 significantly ($p<0.001$) limited the rise in ALT levels after CCl_4 administration (Table 1) (Kumar *et al*., 2013).

Table 1: Effect of Liv.52 (2 Ml/Kg/day, p.o) and *S. nigrum* (2 Ml/100g/day, p.o) for the duration of 10, 20 and 30 days on CCl_4 induced changes in various biochemical parameters (n=6)

Treatment	ALT (IU/l) (Mean ± SD)	ALP (IU/l) (Mean ± SD)	TOTAL BILIRUBIN (mg/dl) (Mean ± SD)
Normal saline (G 1)	25.87 ± 5.30	23.73 ± 11.26	0.25 ± 0.11
CCl_4 (1 ml/kg, ip) (G 2)	423.08 ± 22.02^	237.84 ± 35.83^	1.88 ± 0.21^
Liv.52 + CCl_4 on 20^{th} day (G 3)	105.75 ± 7.06*	67.69 ± 11.63*	0.60 ± 0.07*
S. nigrum x 10 days + CCl_4 on 10^{th} day (G 4)	298.74 ± 19.42*	147.60 ± 16.30*	1.43 ± 0.23*
S. nigrum x 20 days + CCl_4 on 20^{th} day (G 5)	186.64 ± 18.82*	119.46 ± 11.31*	1.25 ± 0.11*
S. nigrum x 30 days + CCl_4 on 30^{th} day (G 6)	104.54 ± 13.90*≠	72.10 ± 14.31*≠	0.80 ± 0.10*≠

^$p<0.001$ as compared to normal saline treated group.

*$p<0.05$ as compared to CCl_4 treated group.

≠$p>0.05$ as compared to Liv. 52 treated group.

Administration of aqueous extract of *S. nigrum* exhibited dose-dependent limitation of alanine transaminase (ALT) rise after CCl_4 administration. A highly significant ($p<0.001$) rise in serum alkaline phosphatase (ALP) levels was seen in CCl_4 treated group as compared to the normal saline treated group. The rise in serum ALP was significantly lower ($p<0.001$) in Liv. 52 treated group after CCl_4 administration as compared to the group which received only CCl_4. The effect of *S. nigrum* treatment on serum ALP levels was dose-related and exhibited a trend similar to that seen in case of ALT. As with ALT, the doses of 2ml/100g for 10, 20 and 30 days of *S. nigrum* produced significantly lesser ($p<0.001$) increments in serum ALP as compared to the CCl_4 treated group. In the doses of 2ml/100g for 30 days, effect of *S. nigrum* on serum ALT and ALP level was comparable to that of Liv. 52 (Kumar *et al*., 2013).

The administration of CCl_4 significantly increased ($p<0.001$) the serum bilirubin as compared to normal saline treated group. The rise in serum bilirubin was significantly low ($p<0.001$) in Liv. 52 treated group after CCl_4 administration as compared to only CCl_4 treated group. *S. nigrum* in the dose of 2ml/100g for 10 days produced less increment of serum bilirubin when compared to CCl_4 treated group ($p<0.05$) while *S. nigrum* in the dose of 2ml/100g for 20 and 30 days ($p<0.001$) showed significant limitation of serum bilirubin rise (Table 1).

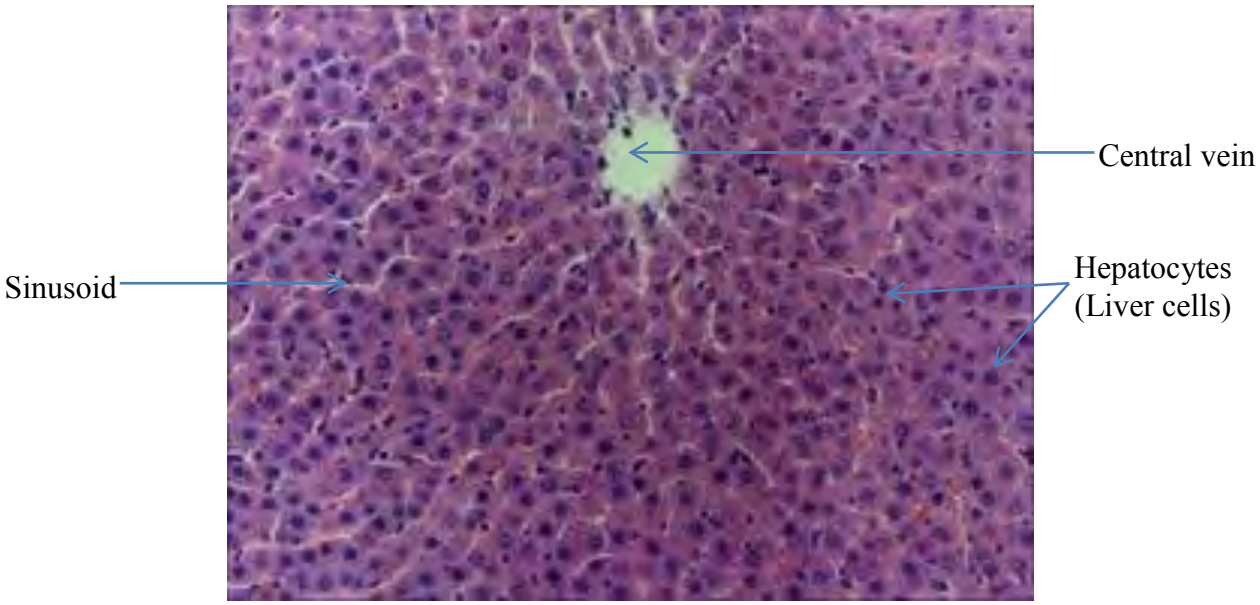


Fig. 2: Histology of normal hepatic lobule (group 1)

Liver cells or hepatocytes are epithelial cells grouped in interconnected plates like the bricks of a wall and the plates are arranged radially around the central vein. Hepatocytes are arranged into thousands of small (~0.7 x 2 mm), polyhedral hepatic lobules which are the classic structural and functional units of the liver. Each lobule has three to six portal areas at its periphery and a venule called a central vein in its center. The hepatic lobule's central vein is actually a venule consisting of little more than an endothelial tube with smaller sinusoids coming in from all directions. Central vein, sinusoids and hepatocytes are normal (Fig. 2).

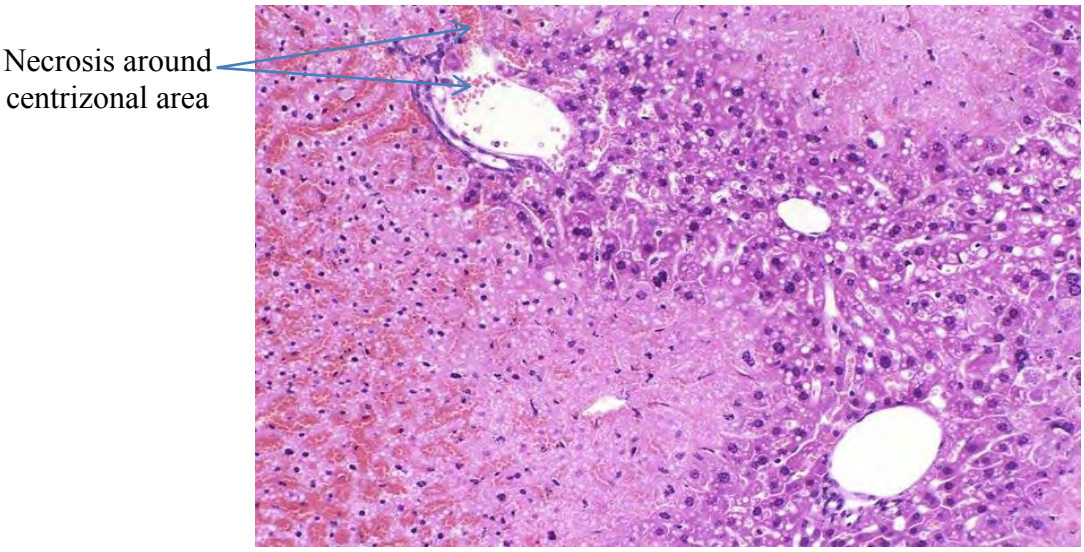


Fig. 3: Microscopic features of the liver of rats treated with CCl_4 (group 2).

Centrilobular necrosis means necrosis around the central zone (called zone 3) and has more P_{450} cytochrome so is more likely to be easily injured. It was seen in the CCl_4 treated group (group 2). The hepatocytes around the central vein were necrosed (Fig. 3).

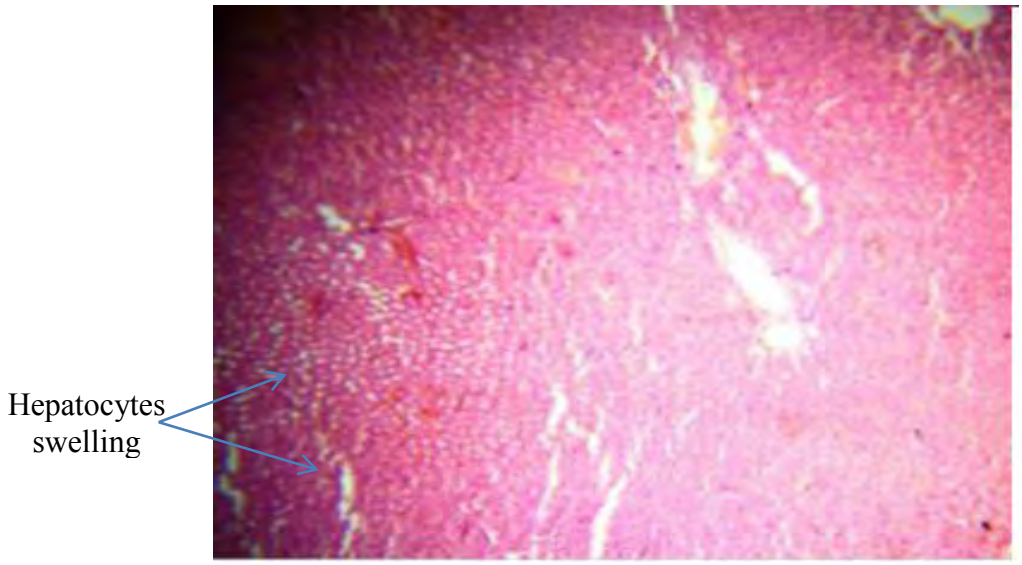


Fig. 4: Microscopic features of the liver of rats treated with Liv.52 (group 3)

Hepatocyte swelling is collection of fluid within the hepatocytes (liver cells) at the site of an injury or infection. This swelling leads something internal change or damage. Liv. 52 treated group revealed very mild signs of liver injury with apparent hepatocyte swelling (Fig. 4).

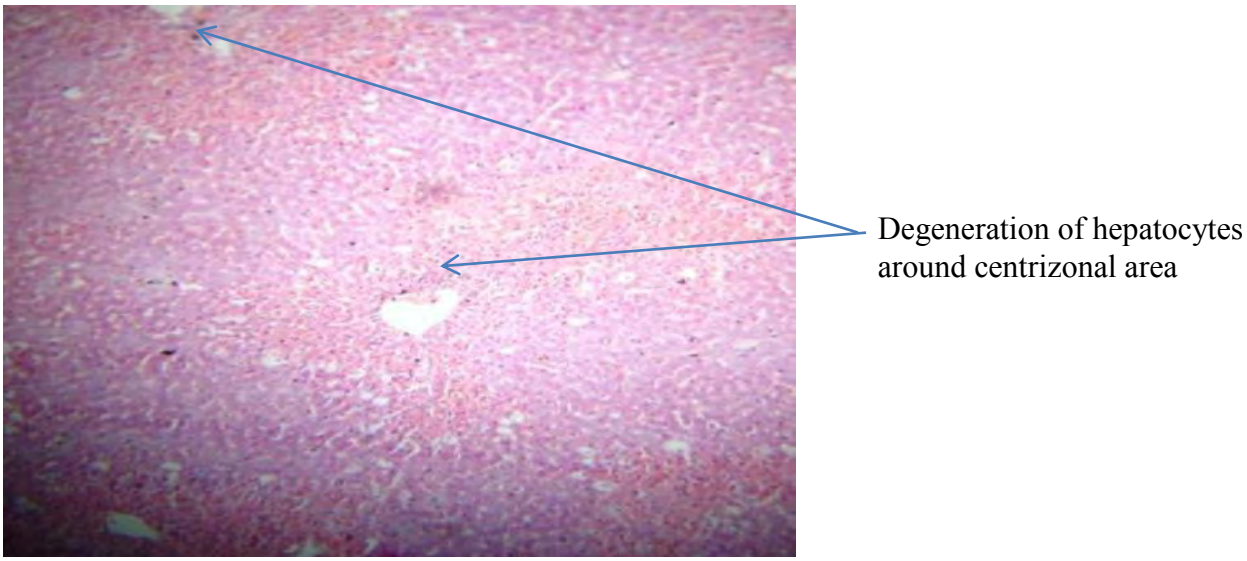


Fig. 5: Microscopic features of the liver of animals treated with S. *nigrum* aqueous extract 2ml/100g/d for 10 days (group 4).

The group treated with aqueous extracts of *S. nigrum* in a dose of 2 ml/100g for 10 days (G 4) showed degeneration in the centrizonal area which was the predominant histological feature of this group (Fig. 5).

Elshater *et al*. (2013) evaluated the hepato-ameliorating effect of *S. nigrum* against CCl_4 induced liver toxicity. The fruits were dried and finely powdered. Thirty two adult male albino rats weighing about 260-300g were divided into four groups (8 rats/cage). Group 1 rats received orally 0.9% NaCl (normal group). Group 2 rats injected with CCl_4 (1 ml/kg), 3 times weekly, for 2 weeks (control group). Group 3 rats injected with CCl_4 (1 ml/kg body weight), 3 times weekly for 2 weeks, following with oral administration of *S. nigrum* extract (500 mg/kg body weight) daily for 30 days (CCl_4 + *S. nigrum* extract). Group 4 rats injected with CCl_4 (1 ml/kg body weight), 3 times weekly for 2 weeks, following oral administration of extract of *S. nigrum* fruits (SNFEt) (250 mg/kg body weight) daily for 30 days (CCl_4 + *S. nigrum* fruits).

At the end of the experiment, all animals were sacrificed and the blood from every animal was taken into clean tubes. The blood, serum and liver tissue were collected for hematological and biochemical analysis, respectively. Liver was removed, cleared off blood and immediately transferred to ice-cold containers containing 0.9% NaCl. Tissues were homogenized in 5ml of the phosphate buffer (K_2HPO_4, $K_2H_2PO_4$, EDTA and PVP) and centrifuged at 4000 rpm for 15min at 4 ºC. Blood was collected in clean dry test tubes with few drops of heparin and plasma obtained was used the examination of complete blood picture (platelets count, red blood cells count (RBCs), leukocytes count (WBCs) and total hemoglobin (Hb) (Elshater *et al*., 2013).

Hb content, PCV value and platelets count were highly significant decreased ($p \leq 0.01$) and RBCs count was significantly decreased ($p \leq 0.05$) while WBCs count was highly significantly increased ($P \leq 0.01$) in control group (CCl_4) as compared with normal group. Hb content and PCV value were significantly increased, however RBCs, platelets count increased in rats treated with both of whole plant extract of *S. nigrum* (G 3) and SNFEt (G 4), while WBCs count was significantly decreased in (G 3) and it decreased in (G 4) when compared with control group (Table 2).

Table 2: Effect of daily oral administration of the extracts of *S. nigrum* (500 mg/kg body weight) and fruits from *S. nigrum* (250 mg/kg body weight) after 30 days of treatment on complete blood picture (RBCs, WBCs, platelets, PCV% value and Hb content) of albino rats, injected with CCl_4 (1 ml/kg b. w.) 3 times weekly for two weeks.

Groups	PCV (%)	Hb Conc. (g/dl)	RBCs ($x10^6/mm^3$)	WBCs ($x10^3/mm^3$)	Platelets ($x10^3/mm^3$)
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Normal (G 1)	48.12 ± 0.64	14.28 ± 0.63	6.02 ± 0.58	7.90 ± 1.07	695 ± 31.62
Control (CCl_4) (G 2)	36.70 ± 1.15	10.38 ± 1.22	4.91 ± 0.62	19.27 ± 4.16	263.50 ± 44.47
CCl_4 + *S. nigrum* extract (G 3)	42.25 ± 2.76	12.72 ± 1.04	5.33 ± 1.67	13.57 ± 2.75	264.13 ± 38.86
CCl_4 + SNFEt (G 4)	46.62 ± 2.5	12.71 ± 1.48	5.88 ± 0.39	15.85 ± 2.64	341.50 ± 2.64

The biochemical results shown that significant decrease in ALT and AST in group treated with CCl_4 and *S. nigrum* extract (G 3), while there was significantly decrease in serum AST and ALT level in group treated with CCl_4 and SNFEt (G 4) as compared with control group. However, serum albumin and total protein were significantly increased in (G 3) and (G 4) when compared with control group (Table 3).

Table 3: Effect of oral administration of daily doses of extracts of *S. nigrum* (500 mg/ kg body weight) and fruits from *S. nigrum* (250 mg/kg body weight) after 30 days of treatment on serum levels of ALT, AST, albumin and protein in albino rats, injected with CCl_4 (1 ml/kg b.w.) 3 times weekly for two weeks.

Groups	ALT (Units/ml)	AST (Units/ml)	Albumin (g/dl)	Protein (g/dl)
	Mean $\pm$ S.D	Mean $\pm$ S.D	Mean $\pm$ S.D	Mean $\pm$ S.D
Normal (G 1)	15.25 $\pm$ 2.05	17.00 $\pm$ 1.92	3.95 $\pm$ 0.09	9.94 $\pm$ 1.07
Control (CCl_4) (G 2)	56.50 $\pm$ 3.07	66.25 $\pm$ 5.20	2.60 $\pm$ 0.26	5.91 $\pm$ 0.29
CCl_4 + *S. nigrum* extract (G 3)	23.37 $\pm$ 2.87	35.00 $\pm$ 4.33	3.77 $\pm$ 0.86	6.70 $\pm$ 0.60
CCl_4 + SNFEt (G 4)	23.62 $\pm$ 2.32	26.93 $\pm$ 2.88	3.70 $\pm$ 0.29	8.76 $\pm$ 0.21

The activities of liver GSH and CAT significantly increased but SOD activity exhibited significant increase in group (G 3) treated with CCl_4 and *S. nigrum* extract, but there was a significant increase in activities of liver GSH and CAT and a significant increase in SOD activity in group (G 4) treated with CCl_4 and SNFEt when compared with control group. The level of liver MDA was significantly decreased in group (G 3) but it showed significant decrease in group (G 4) as compared with control group (Table 4).

Table 4: Effect of daily oral administration of the extracts of *S. nigrum* (500 mg/kg body weight) and fruits from *S. nigrum* (250 mg/kg body weight) after 30 days of treatment on GSH, SOD and CAT activities and level of MDA in liver tissue of albino rats, injected with CCl_4 (1 ml/kg b. w.) 3 times weekly for two weeks.

Groups	SOD (u/g)	CAT (u/g)	MDA (nmol/l)	GSH (mg/g)
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Normal (G 1)	451.05 ± 7.94	1.977 ± 0.01	0.63 ± 0.17	8.31 ± 0.53
Control (CCl_4) (G 2)	341.50 ± 10.32	0.22 ± 0.04	24.94 ± 2.24	0.59 ± 0.14
CCl_4 + *S. nigrum* extract (G 3)	449.87 ± 1.64	1.94 ± 0.03	4.52 ± 1.72	6.06 ± 0.46
CCl_4 + SNFEt (G 4)	440.25 ± 18.35	1.97 ± 0.007	0.68 ± 1.16	7.28 ± 0.59

Mirunalini *et al*. (2012) examined the effect of SNFEt on ethanol induced toxicity in rats. The fruits were washed, shade dried and finely powdered. 100 g of the powder was suspended in 250 ml of water for 2 hours and then heated at 60-65ºC for 30 minutes. The extract was collected separately and the processes were repeated three times with the residual powder, each time collecting the extract. The collected extracts were pooled and passed through fine cotton cloth. The filtrates were evaporated at 40-50ºC in a rotavapour under reduced pressure.

Thirty six adult male albino wistar rats weighing (150-170g) were used for the study. All the animals were acclimatized for a week. The animals were housed in polypropylene cages (45×24×15cm), maintained under the temperature of 25 ± 2ºC and 12 hours light/12 hours dark conditions. The animals had free access to standard pellet diet and water throughout the experimental period and replenished daily. The standard pellet diet comprised of 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorous, 3.4% glucose, 2% vitamin and 55% nitrogen free extract (carbohydrate) and it provided metabolic energy of 3600 kcal/kg (Mirunalini *et al*., 2012).

Animals were randomized into six groups of six animals each. The mode of administration to all the groups was given through gastric intubation. Group 1 rats received 0.2 ml of gum acacia. Group 2 rats received 20% ethanol (3.95 g/kg b.wt twice a day i.e 7.9 g/kg/day). Group 3 rats received 20% ethanol along with SNFEt (250 mg/kg b.wt) on a daily basis for 30 days. Group 4 rats received 20% ethanol along with the standard drug silymarin (25 mg/kg b.wt). Group 5 rats received SNFEt and Group 6 rats received silymarin alone (Mirunalini *et al*., 2012).

At the end of the experimental period (30 days), all the rats were kept fasting overnight and anesthetized using ketamine chloride (24 mg/kg body weight) by intramuscular injection and were sacrificed by cervical decapitation between 8.00 am to 10.00 am. Blood was collected in clean dry test tubes with few drops of heparin and plasma obtained was used for various biochemical results. Tissues such as liver and kidney were removed, cleared off blood and immediately transferred to ice-cold containers containing 0.9% NaCl. Tissues were homogenized in 5ml of the buffer and were used for the estimation of various biochemical parameters (Mirunalini *et al*., 2012).

Body weight was found to be significantly reduced in ethanol treated rats whereas the liver body weight ratio was found to be increased in animals fed with ethanol as compared to control. Supplementation of SNFEt (250 mg/kg b.wt) and silymarin (25 mg/kg b.wt) reversed the weight loss during the experimental period (Table 5).

Table 5: Effect of SNFEt on body weight and liver weight to body weight ratio of control and ethanol administered rats.

Group	Body weight (g)			(Liver wt / bwt) × 100
	Initial (0 day)	Final (30 day)	Net gain (g)	
Control (G 1)	152.78 ± 4.31	176.50 ± 8.41	23.72 ± 4.10	1.98 ± 0.62
Ethanol (G 2)	160.83 ± 7.02	167.00 ± 7.49	6.17 ± 0.47	3.94 ± 1.78
Ethanol + SNFEt (250mg/kg b.wt) (G 3)	157.11 ± 6.26	171.00 ± 5.04	13.89 ± 1.22	2.28 ± 1.05
Ethanol + Silymarin (25mg/kg b.wt) (G 4)	156.21 ± 3.84	175.00 ± 5.66	18.79 ± 1.82	2.17± 0.87
Control + SNFEt (G 5)	153.63 ± 5.77	173.60 ± 7.54	19.97 ± 1.77	2.07 ± 0.68
Control + Silymarin (G 6)	155.00 ± 3.51	175.00 ± 7.96	20.00 ± 4.45	2.00 ± 0.53

Values are expressed as mean ± SD, n=6.

Hepatic markers such as ALP, ALT, AST, GGT and serum bilirubin were significantly elevated in ethanol administered rats. Co-administration of SNFEt significantly reduced the above mentioned markers to near normal levels. This effect was comparable to that observed with the standard drug silymarin. Moreover, there were no significant changes observed in control and treated groups (Table 6).

Table 6: Effect of SNFEt on hepatic marker enzymes and bilirubin in serum of control and experimental animals.

Group	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	GGT (IU/L)	Bilirubin (mg/dl)
Control (G 1)	86.19 ± 5.88	23.94 ± 1.59	74.87 ± 6.56	2.40 ± 0.21	0.59 ± 0.06
Ethanol (G 2)	167.40 ± 14.63	68.15 ± 4.61	146.98 ± 10.47	7.87 ± 1.48	1.72 ± 0.12
Ethanol + SNFEt (250mg/kg b.wt) (G 3)	121.02 ± 7.64	31.62 ± 3.14	108.83 ± 8.43	3.62 ± 0.48	1.03 ± 0.06
Ethanol + Silymarin (25mg/kg b.wt) (G 4)	114.08 ± 9.23	25.06 ± 2.11	90.46 ± 7.83	2.91 ± 0.27	0.85 ± 0.08
Control + SNFEt (G 5)	88.62 ± 3.95	20.66 ± 1.15	76.07 ± 6.02	2.34 ± 0.19	0.58 ± 0.04
Control + Silymarin (G 6)	86.73 ± 6.90	23.55 ± 1.28	75.63 ± 5.33	2.80 ± 0.25	0.60 ± 0.05

Values are expressed as mean ± SD, n=6.

The levels of urea, uric acid and creatinine were significantly higher in ethanol-induced rats whereas treatment with SNFEt and silymarin significantly decreased the levels to near normal values. No significant changes were observed between control and treated groups (Table 7).

Table 7: Effect of SNFEt on renal function markers in the serum of control and ethanol treated rats.

Group	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Control (G 1)	25.38 ± 1.69	1.30 ± 0.06	0.86 ± 0.06
Ethanol (G 2)	45.26 ± 2.96	2.63 ± 0.35	1.88 ± 0.07
Ethanol + SNFEt (250mg/kg b.wt) (G 3)	30.51 ± 2.13	2.01 ± 0.16	0.99 ± 0.08
Ethanol + Silymarin (25mg/kg b.wt) (G 4)	27.86 ± 2.01	1.93 ± 0.13	0.89 ± 0.06
Control + SNFEt (G 5)	23.65 ± 1.19	1.39 ± 0.08	0.80 ± 0.01
Control + Silymarin (G 6)	25.74 ± 1.72	1.35 ± 0.07	0.67 ± 0.03

Values are expressed as mean ± SD, n=6.

The activity of antioxidants (SOD, CAT, GPx and GSH) was remarkably declined in liver of rats administered with ethanol when compared to the other experimental groups. In response to SNFEt and silymarin treatment, the activities were brought back to near normal levels whereas in control and treated groups, no significant changes were indicated (Tables 8).

Table 8: Effect of SNFEt on the activities of SOD, CAT, GPx and GSH in the liver of control and experimental rats.

Groups	Liver			
	SOD (U*/mg protein)	CAT ($U^{\#}$/mg protein)	GPx ($U^{\$}$/mg protein)	GSH (mg/100 g wet tissue)
Control (G 1)	7.48 ± 0.60	74.12 ± 4.15	11.17 ± 2.08	136.57 ± 9.86
Ethanol (G 2)	4.12 ± 0.40	46.32 ± 3.11	5.23 ± 0.16	78.21 ± 6.50
Ethanol + SNFEt (250mg/kg b.wt) (G 3)	6.20 ± 0.45	66.71 ± 4.01	9.09 ± 0.59	95.31 ± 7.92
Ethanol + Silymarin (25mg/kg b.wt) (G 4)	6.43 ± 0.50	67.92 ± 5.60	10.38 ± 1.08	108.87 ± 8.94
Control + SNFEt (G 5)	7.91 ± 0.66	73.46 ± 5.11	10.88 ± 1.29	131.42 ± 9.44
Control + Silymarin (G 6)	7.26 ± 0.59	71.58 ± 4.57	11.57 ± 1.69	134.26 ± 9.67

U*: enzyme concentration required to inhibit the chromogen produced by 50% in one minute under standard condition.

U#: mole of H_2O_2 consumed/minute.

U$: g of GSH utilized/minute.

The activity of antioxidants (SOD, CAT, GPx and GSH) was remarkably declined in kidney of rats administered with ethanol when compared to the other experimental groups. In response to SNFEt and silymarin treatment, the activities were brought back to near normal levels whereas in control and treated groups, no significant changes were indicated (Tables 9).

Table 9**:** Effect of SNFEt on the activities of SOD, CAT, GPx and GSH in the kidney of control and ethanol administered rats.

Groups	Kidney			
	SOD (U*/mg protein)	CAT ($U^{\#}$/mg protein)	GPx ($U^{\$}$/mg protein)	GSH (mg/100 g wet tissue)
Control (G 1)	11.30 ± 1.36	39.82 ± 2.88	8.50 ± 0.50	113.52±9.14
Ethanol (G 2)	7.51 ± 0.74	23.93 ± 2.03	4.77 ± 0.21	63.87±5.57
Ethanol + SNFEt (250mg/kg b.wt) (G 3)	9.77 ± 0.94	34.56 ± 2.28	6.43 ±0.29	96.41±7.81
Ethanol + Silymarin (25mg/kg b.wt) (G 4)	10.02 ± 0.96	37.41 ± 2.42	7.05 ± 0.38	103.62±8.87
Control + SNFEt (G 5)	11.21 ± 1.25	42.29 ± 3.25	9.01 ± 0.68	113.21±9.05
Control + Silymarin (G 6)	11.42 ± 1.45	39.98 ± 3.16	8.14 ±0.47	109.43±8.99

U*: enzyme concentration required to inhibit the chromogen produced by 50% in one minute under standard condition.

$U^{\#}$: mole of H_2O_2 consumed/minute.

$U^{\$}$: g of GSH utilized/minute.

Shaheen *et al*. (2014) evaluated the nephroprotective effects of different doses of aqueous extract of *F. vulgare* seeds, *S. nigrum* fruit and their mixture on gentamicin induced nephrotoxicity in albino rats. A total of 54 animals were divided into 9 groups; each group consisted of 6 animals and they received the treatment as follows:

Group 1 rats were control group.

Group 2 rats were gentamicin-administered (80mg/kg), untreated control.

Group 3 rats received (Gentamicin 80mg/kg + Silymarin 200mg/kg/oral).

Group 4 rats received aqueous extract of *F. Vulgare* 250mg/kg/oral + Gentamicin 80mg/kg.

Group 5 rats received aqueous extract of *S. nigrum* 250mg/kg/oral + Gentamicin 80mg/kg.

Group 6 rats received aqueous extract of mixture 250mg/kg/oral + Gentamicin 80mg/kg.

Group 7 rats received aqueous extract of *F.Vulgare* 500mg/kg/oral + Gentamicin 80mg/kg.

Group 8 rats received aqueous extract of *S. nigrum* 500mg/kg/oral + Gentamicin 80mg/kg.

Group 9 rats received aqueous extract of mixture 500mg/kg/oral + Gentamicin 80mg/kg.

2 ml of blood samples were drawn at 21st day from the jugular vein of rats of all experimental groups. Blood samples were centrifuged at 1507g for 15 minutes. Serum were separated in small aliquots and stored at -20˚C. Concentration of creatinine, urea and albumin were determined from serum samples by using commercially available kits. At 21st day, three animals from each group were sacrificed. Parts of kidneys were embedded in formalin, processed in graded ethanolic concentrations and fixed in paraffin blocks (Shaheen *et al*., 2014).

Phytochemical investigation of *F. vulgare* seeds and fruits of *S nigrum* revealed the presence of various bioactive constituents. Both plants have almost all the major phytoconstituents except glycosides in *S. nigrum* and steroids in *F. vulgare* (Table 10).

Table 10: Preliminary phytochemical analysis of *F. vulgare* and *S. nigrum*.

Phytoconstituents	*F. vulgare*	*S. nigrum*
Tannins	+	+
Saponin	+	+
Terpenoids	+	+
Phenols	++	++
Flavonoids	++	++
Glycosides	±	-
Ascorbic acid	++	++
Free reducing sugar	+	+
Steroids	-	+
Total Sugars	++	++
Alkaloids	+	+

++ Strong positive,

± Trace,

- Negative

Serum creatinine was significantly increased (1.06±0.07 mg/dl) in the GM treated renal injury group, when compared to the control group (0.65±0.01 mg/dl; $P<0.01$). The increase induced by GM was completely attenuated by all the treated groups, displaying their nephroprotective action. However, group 9 had the numerically lowest value of serum creatinine as compared to control group, indicating their improved nephroprotective activity (Table 11).

GM treatment for twenty one days resulted in significant increase in serum urea level compared to control rats. However, elevations in the blood urea were significantly ($P<0.01$) attenuated by all the treated groups indicating their nephroprotective activities. Group 7 and group 9 exhibited more positive nephroprotective effects having significantly ($P<0.01$) lower values of urea level when compared to normal values. Albumin concentration was significantly ($P<0.01$) lower in GM-treated group as compared to normal control group (Table 11).

All the treated groups significantly reversed the lowered values of albumin induced by GM indicating their positive effects to renal damage. However, plants mixture at higher doses (group 9) exhibited substantial higher values of albumin, whereas group 4 and group 5 showed significantly ($P<0.01$) lower values of albumin in comparison to normal control group. Plant extracts alone at higher doses (group 7) and (group 8) also displayed considerable lower concentration of albumin as compared to normal control group (Shaheen *et al*., 2014).

GM treated group revealed significantly ($P<0.01$) lower values of catalase as compared to normal control group. All the treated groups significantly ($P<0.01$) reversed the lower values of catalase induced by GM administration indicating their antioxidant activities. Group 3 had considerable higher concentration of catalase, followed by group 9, group 7, group 8, group 6, group 4 and group 5 respectively as compared to normal control group (Table 11).

Table 11: Effect of *F. vulgare, S. nigrum* and their mixture on serum levels of urea, creatinine, albumin, plasma MDA and CAT in albino rats.

Group	Creatinine (mg/dl)	Urea (mg/dl)	Albumin (g/dl)	MDA (nmol/L)	CAT (KU/L)
Group 1: Normal Control	0.65 ± 0.01	60.63 ± 1.27	4.70 ± 0.14	6.02 ± 0.17	48.14 ± 0.84
Group 2: GN (80mg/kg)	1.06 ± 0.07	85.67 ± 3.73	2.80 ± 0.14	18.36 ± 1.60	28.23 ± 2.10
Group 3: GN + S (80 +200 mg/kg)	0.75 ± 0.04	60.00 ± 2.54	4.50 ± 0.17	6.19 ± 0.22	47.93 ± 0.79
Group 4: GN +FV-I (80 +250mg/kg)	0.75 ± 0.03	68.04 ± 1.80	3.50 ± 0.20	12.07 ± 0.89	36.12 ± 0.93
Group 5: GN + SN-I (80 +250mg/kg)	0.71 ± 0.02	67.58 ± 1.60	3.50 ± 0.19	14.15 ± 1.16	34.01 ± 1.55
Group 6: GN + Mix-I (80 +250mg/kg)	0.68 ± 0.03	62.75 ± 1.83	4.68 ± 0.15	10.25 ± 0.80	37.87 ± 1.56
Group 7: GN +FV-II (80 +500mg/kg)	0.65 ± 0.02	56.50 ± 2.70	4.29 ± 0.15	8.02 ± 0.62	42.04 ± 0.95
Group 8: GN + SN-II (80 +500mg/kg)	0.73 ± 0.07	58.21 ± 2.43	4.24 ± 0.15	10.16 ± 0.60	40.01 ± 1.11
Group 9: GN + Mix-II (80 +500mg/kg)	0.58 ± 0.02	56.21 ± 2.45	5.00 ± 0.20	7.07 ± 0.36	45.80 ± 0.99

GN= Gentamicin, S= Silymarin, FV= *F.Vulgare*, SN= *S. nigrum*, Mix= Mixture

Table 12: Histopathological features as seen in the kidney in the gentamicin model

Histopathological features	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Glomerular congestion	-	+++	-	-	++	+	-	+	-
Peritubular congestion	-	+++	+	-	+	-	-	+	-
Epithelial desquamation	-	+++	-	-	-	+	-	-	-
Blood vessel congestion	-	+++	-	-	-	-	-	-	-
Inflammatory cells	-	+++	-	+	++	-	-	-	-
Necrosis	-	+++	+	+	++	+	+	+	-
Connective tissue proliferation	-	+++	-	+	++	-	-	+	-
Peritubular dilation	-	-	-	-	-	-	++	+	-

(-): normal; (+): little effect; (++): appreciable effect; (+++): severe effect

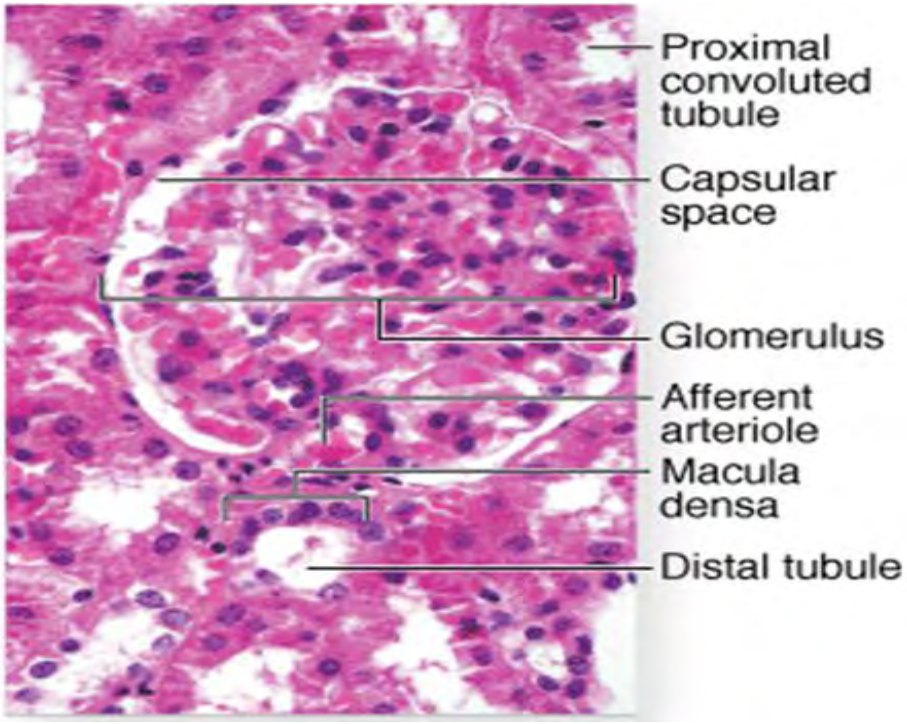


Fig. 6. Microscopic features of renal corpuscle (group 1)

Within each renal lobe are hundreds of thousands of nephrons, the functional units of the kidney. Each nephron originates in the cortex, at the renal corpuscle associated with glomerular capillaries. Extending from the corpuscle is the proximal convoluted tubule, then the nephron loop (of Henle) into the medulla and back to the cortex, then the distal convoluted tubule and collecting tubule which merges into a collecting duct for urine transport to the calyx. At the beginning of each nephron is a renal corpuscle, about 200 m in diameter and containing a loose knot of capillaries, the glomerulus, surrounded by a double-walled epithelial capsule called the glomerular (Bowman's) capsule. Each renal corpuscle has a vascular pole, where the afferent arteriole enters and the efferent arteriole leaves, and a urinary or tubular pole, where the proximal convoluted tubule begins. After entering the renal corpuscle, the afferent arteriole usually divides and subdivides into the two to five capillaries of the renal glomerulus (Fig. 6).

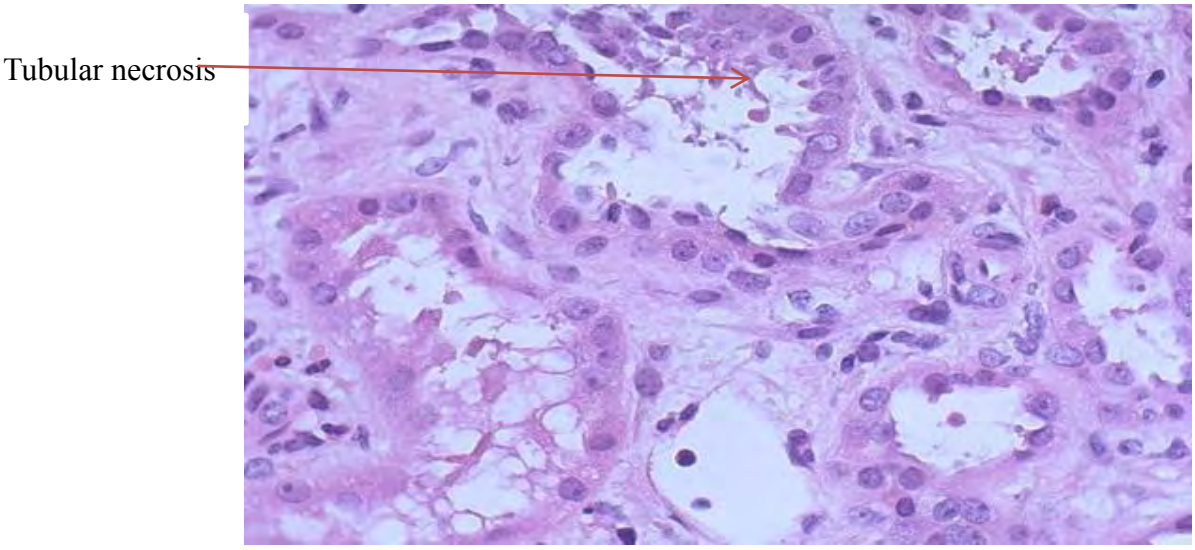


Fig. 7. Microscopic features of nephron of rats treated with gentamicin (group 2).

The microscopic features of kidney of rats treated with gentamicin conformed tubular vacuolization and tubular dilation are the result of the toxic effect of gentamicin (Fig. 7).

2.2. Toxic effects of *S. nigrum* L.

According to Ganguly *et al.* (2009), *S. nigrum* is generally regarded as a toxic plant. In a typical experiment, dried and powdered whole plant material of *S. nigrum* was hot extracted with petroleum ether followed by hot aqueous methanol (40:60) (Soxhlet). Solvent was evaporated from the methanol extract, under reduced pressure, and the residue was partitioned between *n*-butanol and water.

Swiss albino mice of both sex and approximately the same weights were divided into ten groups for each extract, each containing six animals for the purpose of determining the LD50 values of *S. nigrum* and metal ions conjugated (*S. nigrum*/glycoalkaloids) and metal ions depleted glycoalkaloids of *S. nigrum* (*S. nigrum*/glycoalkaloids/excluded). After 24 hours of test compound administration, the number of dead animals in a group was recorded. The toxicological effect was assessed on the basis of mortality, which was expressed as an LD_{50} value (Ganguly *et al.*, 2009).

For subacute oral toxicity study, albino rats weighing 200-250g were divided into 10 groups of six animals each.

Group 1 rats received only distilled water.

Group 2 rats received *S. nigrum* 1g/kg body weight.

Group 3 rats received *S. nigrum* 2g/kg body weight.

Group 4 rats received *S. nigrum* 4g/kg body weight.

Group 5 rats received *S. nigrum*/glycoalkaloids 100 mg/kg body weight.

Group 6 rats received *S. nigrum*/glycoalkaloids 200 mg/kg body weight.

Group 7 rats received *S. nigrum*/glycoalkaloids 400 mg/kg body weight.

Group 8 rats received *S. nigrum*/glycoalkaloids/excluded 100 mg/kg body weight.

Group 9 rats received *S. nigrum*/glycoalkaloids/excluded 200 mg/kg body weight.

Group 10 rats received *S. nigrum*/glycoalkaloids/excluded 400 mg/kg body weight. The rats were treated with the drugs for 21 consecutive days (Ganguly *et al.*, 2009).

The *S. nigrum* total extract was safe if less than 5 g/kg body weight. This is presumably due to the fact that glycoalkaloids of *S. nigrum* contain appreciable amount of conjugated metal ions (except Cu^{2+}). Metal-free excluded fractions were prepared by passing it through a strong cation exchange resin (Dowex 50/H+); this product (metal-free) was found to be more toxic than the total glycoalkaloids, as indicated by the LD_{50} values (Table 13).

Table 13: Determination of LD_{50} value of different fractions *S. nigrum*

Samples	LD_{50} in g/kg body weight
S. nigrum	Less than 5.0
S. nigrum/glycoalkaloids	2.0
S. nigrum/glycoalkaloids/excluded (metal ions depleted fraction)	1.6

Note: The toxicity in the metal ions depleted fraction of glycoalkaloids was increased considerably as indicated by lower LD_{50} value.

The increasing trend in toxicity of the metal ions depleted glycoalkaloids of *S. nigrum* was further manifested in the sub-acute toxicity study. The total extracts of *S. nigrum* was found to be safe, as revealed by the hematological and hepatic parameters, up to a dose of 4 gm/kg, p.o. administered for 21 days as compared to normal control animals. Whereas glycoalkaloids fraction of *S. nigrum* was found to be toxic at a dose of 200 and 400 mg/kg, p.o. treated for 21 days. The toxicity of the glycoalkaloids was further increased when the extract was made metal ions depleted by passing through a strong cation exchange resin (Table 14).

Table 14: Sub-acute toxicity study of *S. nigrum* total extracts and glycoalkaloids of *S. nigrum* with and without conjugated metal ions

Groups	Hematological and hepatic parameters on 21st day						
	Total RBC Count (x106/ µl)	Total WBC Count (x103/ µl)	Hb (g/dl)	AST (in U/L)	ALT (in U/L)	ALP (in U/L)	Total protein in mg/dl
Normal control	7.30±0.39	5.51±0.38	14.35±0.49	64.06±2.72	56.25±2.24	19.31±1.40	7.62±0.83
S. nigrum /1gm/kg	7.36±0.47	5.68±0.38	14.50±0.51	62.04±1.71	54.62±2.92	19.93±2.01	7.75±0.61
S. nigrum /2gm/kg	7.29±0.27	5.72±0.39	14.71±0.95	63.29±2.13	54.32±2.68	19.71±1.52	8.41±1.02
S. nigrum /4gm/kg	7.52±0.69	5.59±0.36	14.68±0.39	63.55±1.99	51.51±2.08	19.01±1.35	7.91±0.93
S. nigrum /glyco- 100mg/kg	6.99±0.18	5.70±0.40	13.99±0.49	64.97±2.78	57.28±2.16	19.51±0.43	7.27±0.69
S. nigrum /glyco- 200mg/kg	6.83±0.33*	5.90±0.37	13.55±0.35*	68.35±2.22*	59.68±1.49*	19.88±0.39*	6.92±0.57
S. nigrum /glyco- 400mg/kg	6.67±0.26**	6.15±0.46*	12.93±0.36**	68.86±2.54*	62.57±2.04**	21.27±0.61**	6.16±0.49**
S. nigrum /glyco/excluded 100mg/kg	6.87±0.21*	6.04±0.40	13.81±0.51	67.27±2.91	58.62±2.25	19.67±0.36*	7.15±0.79
S. nigrum /glyco/excluded 200mg/kg	6.61±0.28**	6.19±0.34*	13.29±0.34**	68.35±2.37*	61.84±1.71**	20.28±0.56**	6.56±0.54*
S. nigrum /glyco/excluded-400mg/kg	6.49±0.17**	6.35±0.38**	12.47±0.28**	69.63±2.28**	63.84±2.35**	22.22±0.88**	5.78±0.44**

Data represented as Mean + SD; for 6 rats. *p<0.05; ** p <0.01; in comparison to Group 1 (normal control) rats treated with the vehicle.

3. Discussion

S. nigrum L. is one of the potential medicinal plants that is used to treat both human and livestock ailments all over the world. In addition, this plant has pharmacologic significance and is an edible plant in some parts of the world.

Various research findings showed that *S. nigrum* has different chemical compounds such as alkaloids, saponins, flavonoids and tannins that are used for both hepatoprotective and nephroprotective functions (Maharana *et al*., 2011; Elias *et al*., 2013; Senthilnath *et al*., 2013; Shaheen *et al*., 2014). In addition, the pharmacological constituents separated out from *S. nigrum* are steroidal glycoalkaloids (solamargine, solasonine, solanigrine), steroidal genin (titogenin), saponins and vitamins (riboflavin, nicotinic acid, ascorbic acid and carotene). These constituents are responsible for diverse activities including anti-inflammatory, anti-microbial, anti-helminthic and antifungal activities (Kumar *et al*., 2013).

The liver damage induced by CCl_4 is commonly used model for the screening of hepatoprotective drugs. Because the CCl_4 induced liver of rats resulted from its reductive dehalogenation (metabolically activated) by the cytochrome p-450 dependent mixed oxidase enzyme system in endoplasmic reticulum to form trichloromethyl free radical, which readily interacts with molecular oxygen to form the trichloromethyl peroxy free radical (Williams and Burk, 1990). Both free radicals (trichloromethyl and trichloromethyl peroxy) are capable of binding to proteins or lipids leading to membrane lipid peroxidation and finally cell necrosis (DeGroot and Noll, 1986). This is evidenced by Kumar *et al*. (2013) histologically the liver treated with CCl_4 showed severe centrizonal necrosis, necrosis of hepatocytes around central vein with no distinguishable nuclei.

The CCl_4 induced liver of rats is treated with aqueous extracts of *S. nigrum* in a dose of 2 ml/100g for 10 days and showed feathery degeneration in centrizonal area. *S. nigrum* extract has the ability to maintain the structural integrity of hepatocytic cell membrane and is involved in the regeneration of damaged liver cells (Hsieh *et al*., 2008; Lin *et al*., 2008; Kumar *et al*., 2013).

According to Elshater *et al*. (2013) the liver of rats injected with CCl_4 (1 ml/kg body weight) resulted in a decreased PCV, Hb levels, platelets and RBCs counts whereas increased WBCs count as compared to the normal group. The depression in RBCs count and Hb content could be attributed to disturbed hematopoiesis, destruction of erythrocytes, reduction in the rate of their formation and /or their enhanced removal from the circulation (Essawy *et al*., 2010). The elevation of WBCs may be attributed to the defensive mechanism of the immune system. So the activities of free radicals in the liver of rats that lead to increase the WBCs count indicated that there is liver damage due to CCl_4 injection (Oluyemi *et al*., 2007).

Administration of both the two extracts of *S. nigrum* (whole plant extract and fruits extract) altered these changes i.e., elevated the PCV, Hb levels, RBCs and platelets count and slightly decreased the WBCs. It may be due to the presence of active constituents present in *S. nigrum* of whole plant and fruit extracts which stimulating the maturation and development of RBCs which in turn increased the level of Hb and PCV (Vigila and Baskaran, 2011). There is high content of ascorbic acid in *S. nigrum* which plays an important role in absorption and transport of iron. So it supplies iron for development and maturation of RBCs (Elshater *et al*., 2013). This is in contrast to Ganguly *et al*., (2009) stated that conjugated metal ions decreased hematological parameters such as RBCs and Hb and the WBCs increased when treated with more than 100 mg/kg extract of *S. nigrum*. This may indicate that the extract of *S. nigrum* form integration with metal ions or activate in a dose-dependent manner.

The free radicals (trichloromethyl and trichloromethyl peroxy) also resulted in structural changes of endoplasmic reticulum and membranes of other organelles, loss of metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphatase activation, leading to liver injury and elevated levels of ALT, ALP, bilirubin (Gravel *et al*., 1979; Wolf *et al*., 1980; Azri *et al*., 1992).

During liver injury the function of hepatocyte transport is disturbed which leads to leakage of plasma membrane, thereby causing an increased enzyme level in serum (Jadon *et al*., 2007). Liver function tests are useful in identifying hepatic dysfunction (Martin-Lopez *et al*., 1993; Astegiano *et al*., 2004; Thapa and Walia, 2007; Kim, 2008). Since liver executes a variety of functions, no single test is adequate to offer complete estimate of liver functions (Astegiano *et al*., 2004; Kim, 2008).

The biochemical markers of serum ALP, ALT and bilirubin were found to be significantly elevated after CCl_4 administration (Visweswaram *et al*., 1994; Jayasekhar *et al*., 1997). This could be explained by the fact that bilirubin reaches peak serum level in the second hour after CCl_4 administration but this level would have declined afterwards due to treatment with extract of *S. nigrum* (for 10, 20 and 30 consecutive days) (Kumar *et al*., 2013). This is similar to the findings of Sathiavelu *et al*. (2009) and Mirunalini *et al*. (2012) that showed significant increase in the activities of liver marker enzymes such as ALP, ALT and bilirubin on ethanol intoxicated rats, indicated increased permeability, damage and necrosis of hepatocytes. However, treatment of the ethanol injected rats with SNFEt results in a significant reduction of the increased activities of liver marker enzymes to near normal levels which may be a consequence of stabilization of plasma membrane, maintaining the functional and protective effect of *S. nigrum* on liver.

The above idea is also similar to Elashater *et al*. (2013) because injection of CCl_4 (1ml /kg body weight) increased serum ALT activities as compared with normal values, reflecting the damage of the liver cells or changes in the cell membrane permeability leading to leakage of the enzyme from cells to the circulation (Botsoglou *et al*., 2008). Administration of *S. nigrum* extract against this significantly decreased the elevated enzyme. The reduction of ALT level toward the normal value indicated that *S. nigrum* play an important role in anti-lipid peroxidation, stabilization of plasma membranes as well as repair of hepatic tissue damage of free radicals produced by CCl_4 (Lin *et al*., 2008; Feroz *et al*., 2013).

According to Elashater *et al*. (2013) injection of CCl_4 (1ml /kg. body weight) resulted in decreased serum albumin and total protein as compared to normal values. The diminution of total protein and albumin is due to liver damage induced by CCl_4 (Botsoglou *et al*., 2008). Administration of *S. nigrum* extract significantly increased serum albumin and total protein. This may be due to the presence of active constituents such as flavonoids and alkaloids which may prevent the excessive break down of protein (Vigila and Baskaran, 2011).

This assertion was in agreement with Ganguly *et al*. (2009) who showed that total protein in normal control group were 7.62 $\pm$ 0.83mg/dl when treated with 1gm/kg and 2mg/kg of *S. nigrum* extract it was elevated to 7.75 $\pm$ 0.63mg/dl and 8.41 $\pm$ 1.02mg/dl respectively. However, when treated with 100mg/kg and 400mg/kg of *S. nigrum* extract the total protein was reduced to 7.27 $\pm$ 0.69mg/dl and 5.78 $\pm$ 0.44mg/dl. This may indicate that the activity of extract of *S. nigrum* in a dose dependent manner.

In addition, this is in the same stripe with Kibichiy *et al*. (2013) who indicated that there was a general reduction in the levels of albumin concentrations among the infected with *Trypanosoma brucei rhodesiense* parasites mice during the experimental period. Mice treated with 10g/L and 6.7g/L extract of *S. nigrum* had a significantly higher decline in the albumin concentrations over time ($p<0.05$). However, the mice treated with 3.3g/L had a slight decline in the albumin concentrations which however was statistically significant ($p<0.05$) when measured at day 50 post infection. The decline in the concentrations of albumin in dexamethasone treated mice and untreated mice was also significantly lower when compared to those treated with 3.3g/L but higher when compared to the mice treated with 6.7g/L and 10g/L *S. nigrum* extract.

According to Mirunalini *et al*. (2012) the activity of antioxidants such as SOD, CAT, GPx and GSH remarkably declined in liver of rats administered with ethanol when compared to the other experimental groups. When treated with *S. nigrum* extract and SNFEt the activities are brought back to near normal levels whereas in control and treated groups, no significant change was indicated. This is similar to Elashater *et al*. (2013) who indicated that injection of CCl_4 produced oxidative stress as evidenced by a significant decreased in SOD, CAT and GSH activities but increased MDA level. Treatment with *S. nigrum* increased SOD, CAT and GSH level while it decreased MDA level. High content of antioxidants contribute to free radical scavenging activities. Moreover, the whole plant and SNFEt exhibited a potent hepato-ameliorating and antioxidant effects in CCl_4-induced hepatotoxic rats. But hepato-ameliorating and antioxidant effects of extract of *S. nigrum* fruits were found to be better than those of extract from whole plant of *S. nigrum* (Lin *et al*., 2008).

Kidney is an important organ actively involved in maintaining homeostasis of the body by reabsorbing important material and excreting waste products. It has been reported that habitual consumption of large amount of alcohol is associated with an increased risk of kidney failure in the general populations (Parekh and Klag, 2001). Kidney functional markers such as urea, uric acid, creatinine and CAT are the main indicators of renal dysfunction (Mirunalini *et al*., 2012).

According to Shaheen *et al*. (2014), in normal control group the serum creatinine (0.65 $\pm$ 0.01mg/dl) and urea (60.63 $\pm$ 1.27mg/dl) were significantly increased to 1.06 $\pm$ 0.07mg/dl and 85.67 $\pm$ 3.73mg/dl respectively while CAT was reduced from 48.14 $\pm$ 0.84mg/dl to 28.23 $\pm$ 2.10mg/dl in GM treated renal injury. When the GM treated renal injury further treated with *S. nigrum* extract resulted in reduction of serum creatinine (0.71 $\pm$ 0.02mg/dl) and urea (67.58 $\pm$ 1.60mg/dl). However, the level of CAT was elevated to 34.01 $\pm$ 1.55mg/dl.

This is in agreement with Mirunalini *et al*. (2012) who indicated that the normal control levels of urea (25.38 ± 1.69mg/dl), uric acid (1.30 ± 0.06mg/dl) and creatinine (0.86 ± 0.06mg/dl) in ethanol-induced rats were significantly increased to 45.26 ± 2.96mg/dl, 2.63 ± 0.35mg/dl, 1.88 ± 0.07mg/dl respectively whereas treatment with SNFEt (250mg/kg b.wt) significantly decreased 30.51 ± 2.13mg/dl, 2.01 ± 0.16mg/dl, 0.99 ± 0.08mg/dl the levels to near normal values. This may indicate the extract of *S. nigrum* and SNFEt improved nephroprotective activity.

The antioxidants in normal control group such as SOD (11.30 ± 1.36U*/mg protein), CAT (39.82 ± 2.88U$^{\#}$/mg protein), GPx (8.50 ± 0.50U$^{\$}$/mg protein) and GSH (113.52 ± 9.14mg/100g) was remarkably declined to SOD (7.51 ± 0.74U*/mg protein), CAT (23.93 ± 2.03U$^{\#}$/mg protein), GPx (4.77 ± 0.21U$^{\$}$/mg protein) and GSH (63.87 ± 5.57mg/100g) in kidney of rats administered with ethanol when compared to experimental groups. In response to SNFEt the activities were brought back to near normal levels, SOD (9.77 ± 0.94U*/mg protein), CAT (34.56 ± 2.28U$^{\#}$/mg protein), GPx (6.43 ± 0.29U$^{\$}$/mg protein) and GSH (96.41 ± 7.81mg/100g) (Mirunalini *et al*., 2012). This increase could be due to efficient scavenging of ROS which might be implicated to oxidative activation of enzymes (Jayaraman *et al*., 2009). SNFEt preserves the functional capacity of the kidney against ethanol toxicity.

According to Shaheen *et al*. (2014) the histopathological features as seen in the kidney in the gentamicin induced resulted in severe effect of glomerular congestion, peritubular congestion, epithelial desquamation, blood vessel congestion, inflammatory cells, necrosis and connective tissue proliferation. Increase in intracellular free oxygen radicals can initiate irreversible cellular injury process leading to tubular necrosis and tubular degeneration in renal tissues. Scavenging of free oxygen radicals prevent irreversible renal cell injury and necrosis. Many studies confirmed that mediation of ROS may have linked with degenerative tubular effects of gentamicin (Walker *et al*., 1999).

Researchers like Ganguly *et al.* (2009) stated the *S. nigrum* total extract was safe if less than 5 g/kg body weight. This is presumably due to the fact that glycoalkaloids of *S. nigrum* contain appreciable amount of conjugated metal ions (except Cu^{2+}). The increasing trend in toxicity of the metal ions depleted glycoalkaloids of *S. nigrum* further manifested in the sub-acute toxicity study. The *S. nigrum* total extract was found to be safe in the hematological and hepatic parameters, up to a dose of 4 gm/kg, p.o. administered for 21 days as compared to normal control animals. Whereas glycoalkaloids fraction of *S. nigrum* was found to be toxic at a dose of 200 and 400 mg/kg, p.o. treated for 21 days.

This is analogous to Feroz *et al.* (2013) stated that the normal dose of *S. nigrum* (0.43 ml/kg) possessed hepatoprotective effects against CCl_4 induced liver damage in rats. Further the microscopic examination of hepatic tissue in animals kept on normal and moderate dose (5ml/kg) of herbal drug showed inflammatory changes, whereas animals kept on high dose (10ml/kg) showed mild patchy necrosis as compared to control animals. This may be due to the toxicity effects of *S. nigrum* extract at high doses.

4. Conclusion

S. nigrum is commonly called black night shade that belongs to Solanaceae family. Traditionally it is frequently used to treat human health problems. This plant has various bioactive constituents such as alkaloids, flavonoids, polyphenols, solanines, saponins, and tannins that are important for hepatoprotective and nephroprotective functions. These constituents are responsible for diverse activities including anti-inflammatory, anti-microbial, anti-helminthic and antioxidant activities.

This review showed that *S. nigrum* whole plant extract and SNFEt were able to reverse the pathological parameters (serum levels of ALP, ALT, AST and bilirubin) of liver and kidney to near normal levels. This plant also showed free radical scavenging capacity, liver and kidney regeneration which are very important for human health. This protecting and regeneration capacity may be due to having at least partially to high content of the above mentioned bioactive constituents in *S. nigrum* extract.

The *S. nigrum* whole plant extract and SNFEt were found to be safe for the liver and kidney parameters up to 5ml/kg dose having good protective activities and it was shown that this were dose-dependent activities. But this would have been toxic at elevated doses. Safe dosage needs to be identified for children and pregnant women because children have less body resistance and pregnant women may abort since it may induce uterine contraction.

Therefore, further studies are required to isolate the active ingredients from the extract of *S. nigrum* for proper drug development to treat the above mentioned health problems by conducting further clinical trials.

5. References

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