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Nephroprotective activity of aqueous extract of *Solanum nigrum* in Amphotericin B induced Wister rats

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This study was investigated the nephroprotective activity of *Solanum nigrum* in Amphotericin B (AmB) induced nephrotoxicity in Wister Rats. The nephroprotective effect was analyzed by biochemical parameters such as haematological, biochemical parameters, antioxidants activity. The result shows that the hematological parameters such as RBC, WBC, Hb, PCV and platelet count were significantly decreased in AmB induced nephrotoxic rats. Biochemical parameters such as urea, BUN, uric acid and creatinine levels were significantly increased, total protein and albumin level were significantly reduced in AmB induced nephrotoxic rats. Enzymatic antioxidants and non-enzymatic antioxidants levels were significantly decreased in nephrotoxic rats. Pretreatment with aqueous extract of *S. nigrum* prevent the biochemical changes which near to normal suggest that *S. nigrum* has nephroprotective activity.

Solanum nigrum/ Nephrotoxicity

Our body is always exposed to toxic organic compounds from both intentional and unintentional sources. Drug toxicity is occasionally responsible for kidney complications, although toxicity issues generally resolve when the drug in question is discontinued. The immunosuppressive drug may cause constriction of the blood vessels in the kidneys and thereby alter kidney function. Systemic fungal infections are common and are difficult to treat successfully. In humans, the most common and dose limiting side effect of Amphotericin B is severe nephrotoxicosis (Perfect et al. 1991; Carlson and Condon 1994). The actual mechanism of action may be more complex and multi-faceted. The antifungal activity of AmB is dependent on the drugs binding to the cell membrane sterols. AmB binds more avidly to ergosterol, the principal sterol in fungal membranes. By binding to ergosterol, AmB causes pores or channels to form in the fungal cell membrane, allowing leakage of a variety of small molecule with eventual cell death (Lampen, 1969; Gale 1984; Yu, 1998). Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin that causes damage to kidneys. It involves reduction of renal blood flow and glomerular filtration rate (Sabar, 1990).

Solanum nigrum is used as medicine and possess greater narcotic properties. In India, *S. nigrum* mixed with other herbal medicine has hepatoprotective effect in cirrhotic patients. Protective effect can be attributed to the diuretic, anti-inflammatory, anti-oxidative and immuno modulating properties of the component herbs (Fallah Husein, 2005). It also protects against hepatitis B virus infection (De Silva, 2003; Galitskii 1997; Kalab and Krechler, 1997). The objective of our present study is to evaluate the qualitative phytochemicals screening and nephroprotective activity of aqueous extract of *Solanum nigrum.*

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Materials and Methods

Extraction

The dried powder of *S. nigrum* was weighed and transferred to a clean conical flask containing different solvents such as petroleum ether, benzene, diethyl ether, chloroform, ethyl acetate, acetone, methanol, ethanol, butanol and water and kept in shaker separately for 24 hours in airtight condition. After 24 hrs, it was filtered using Whatmann No.1 filter paper and centrifuged. The supernatant was collected and evaporated under dark room, till the solid yield of extract was obtained. The resulting residue was further used (Hui-Mei et al. 2008).

Phytochemical screening

The phytochemical compounds were qualitatively analyzed by standard method (Brindha et al. 198). Based on the results of the phytochemical analysis, aqueous extract was selected for the present study.

Selection of the animals

Adult male Wister Albino rats were used in the experimental study. The animals were purchased from Perundurai Medical College, Erode. Adult male rats of 12 weeks old weighing about 100–150 g were selected. They were housed in polypropylene cages. Husk was renewed every 24 hrs. The animals were fed with a standard diet (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*. The rats were maintained at the standard animal house conditions. After randomization into various groups, the rats were acclimatized to the laboratory conditions of temperature and photoperiod for a period of 1–2 weeks before initiation of the experiments.

Experimental group

Each group consists of 6 animals. Group I serve as normal [control], Group II serve as drug induced control [AmB 10 mg/kg body weight], Group III serve as concentration I [100 mg of *S. nigrum* extract/kg of body weight + drug induced (10 mg AmB/kg body weight)], Group IV serve as concentration II [200 mg of *S. nigrum* extract/kg of body weight + drug induced (10mg AmB/kg body weight)], Group V serve as positive control [100 mg of *S.nigrum* extract/kg body weight alone] (Hui-Mei et al. 2008).

Administration

The dried residue of *S. nigrum* was weighed and dissolved in sufficient quantity of water at a dose of 100 and 200 mg/kg of body weight. The solution was administered orally to the rats to study its effect. 1 ml of each concentration of the *S. nigrum* aqueous extract was given to the respective groups orally for 30 days (pretreatment). At 25th day, the nephrotoxicity was induced by injecting AmB intraperitoneally to the rats of corresponding groups at 10 mg/kg animals. The doses of AmB (Kavlock et al. 1985; LeBurn et al. 1986) are obtained in consultation with the literature.

Collection of blood, serum and tissue samples

At the end of the 31st day, animals were decapitated and blood was collected in two tubes. The first tube contained calcium EDTA for complete blood analysis. Blood sample in the other tube was left for a short time to allow clotting. Clear serum samples were obtained by centrifugation at 3000 rpm for 20 min and then kept in the refrigerator for further assay. Tissues were immediately excised, immersed in ice-cold physiological saline, dried, weighed and homogenized in ice-cold double distilled water. A suitable aliquot of different homogenates was mixed with Tris–HCl buffer (0.01 mol/l, pH 7.4) to make 10% homogenate and centrifuged at 10,000×*g* at 4°C for 30 min. The resulting supernatant was used for the estimation of antioxidant enzymes.

Statistical analysis

Values were expressed as mean ± SD for 6 animals in each group. The difference between two groups was determined using student's *t* test. The significance was considered as $P<0.05$.

Results and discussion

Phytochemical analysis

Table 1 summarized that the phytochemical constituents present in petroleum ether, benzene, diethyl ether, chloroform, ethyl acetate, acetone, methanol, ethanol, butanol and water extracts of *solanum nigrum*. Due to low polarity, only three components such as alkaloids, flavanoids and cardiac glycosides are present in petroleum ether extract. The water extract showed the presence of alkaloids, flavanoids, steroids, glycosides, saponin, tannin, phenolic compound, thiols, triterpenoids and anthroquinone.

Nephroprotective activity: *in vivo* model

Anemia is virtually a constant symptom of AmB induced nephrotoxicity

(Ceylan et al. 2003). Anemia is due to haemolysis which increases the RBC break down; it will decrease the RBC level (Yu et al. 1998). AmB is highly haemolytic which induces the excessive breakdown of RBC which decreases the level of RBC in blood. There is high content of ascorbic acid in *S. nigrum* (Mahanom et al. 1999). Ascorbic acid plays an important role in iron absorption and its transport. It increases the iron transport and supplies iron for development and maturation of RBC. AmB induces bone marrow suppression which is the site of RBC and WBC development (Ceylan et al. 2003). WBC plays an important role in immune response. Due to nephrotoxicity, WBC count was decreased which may leads to immune deficiency diseases. The active constituents present in *S. nigum* increases WBC development, but the exact mechanism is not known.

Table - 1: Preliminary phytochemical analysis of *Solanum nigrum* extracts

Solvents	Alkaloids	Flavonoids	Steroids	Cardiac glycosides	Saponins	Tannins	Phenols	Thiols	Resins	Triterpenoids	Anthro Quinone
Petroleum ether	+	+	–	+	–	–	–	–	–	–	–
Benzene	+	+	+	_	–	+	+	–	–	+	+
Diethyl ether	+	+	+	+	–	–	+	–	+	+	+
Chloroform	+	+	+	–	–	–	+	–	–	–	–
Ethyl acetate	+	+	–	+	–	–	+	–	–	–	–
Acetone	+	+	+	++	–	+	+	–	+	+	–
Methanol	–	++	+	++	+	–	+	–	+	+	–
Ethanol	+	+	–	+	+	–	+	–	–	–	+
Butanol	+	+	–	+	+	–	+	–	–	–	+
Water	+	+	+	+	+	+	+	+	–	+	+

(++)- Dark colour; (+) - Presence; (–)- Absence

Table - 2: Effect of aqueous leaf extract of *S. nigrum* pretreatment on RBC, WBC, Hb, PCV and platelet count in normal and experimental rats

Animal Groups	RBC (cells/mm^3)	WBC (cells/mm^3)	Hb (g/dl)	PCV (%)	Platelet (cells/mm^3)
Group-I	7.65 ± 0.15	12.65 ± 0.05	14.10 ± 0.60	39.90 ± 0.1	227.8 ± 2.1
Group-II	4.97 ± 0.13	11.25 ± 0.05	11.00 ± 0.10	37.80 ± 0.9	211.5 ± 0.50
Group-III	6.51 ± 0.08	12.88 ± 0.18	13.42 ± 0.18	40.92 ± 0.21	221.4 ± 0.81
Group-IV	6.54 ± 0.14	12.94 ± 0.21	13.51 ± 0.19	39.12 ± 0.31	223.1 ± 0.94
Group-V	6.79 ± 0.12	12.91 ± 0.49	13.49 ± 0.18	39.12 ± 0.34	223.9 ± 0.31

Table - 3: Effect of aqueous leaf extract of *S. nigrum* pretreatment on blood urea and BUN, blood uric acid, creatinine and serum protein and albumin in normal and experimental rats

Animal Groups	Urea (mg/dl)	BUN (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Protein (g/dl)	Albumin (g/dl)
Group-I	45.5 ± 0.21	22.3±0.2	3.74 ± 0.01	0.91 ± 0.01	6.65 ± 0.13	3.84 ± 0.02
Group-II	67.3 ± 0.21	31.3±0.15	4.93 ± 0.02	1.43 ± 0.02	4.94 ± 0.02	2.49 ± 0.17
Group-III	62.3 ± 0.1	29.3±0.21	4.42 ± 0.01	1.31 ± 0.05	5.57 ± 0.02	3.08 ± 0.03
Group-IV	50.2 ± 0.1	26.3±0.2	3.93 ± 0.01	1.21 ± 0.02	5.70 ± 0.01	3.19 ± 0.01
Group-V	49.3 ± 0.21	27.2±0.15	3.64 ± 0.01	1.03 ± 0.06	6.79 ± 0.06	3.82 ± 0.02

Table - 4: Effect of aqueous leaf extract of *Solanum nigrum* pretreatment on enzymatic antioxidants activity in normal and experimental rats

Animal Groups	GPx (U/mg protein)	GR (U/mg protein)	GST (U/mg protein)	CAT (U/mg protein)	SOD (U/mg protein)
Group-I	4.72±0.32	4.23±0.74	859.87±0.82	3.19±0.24	3.25±0.35
Group-II	0.86±1.53	2.75±0.50	625.56±0.43	0.36±0.43	1.25±2.9
Group-III	3.04±1.73	3.69±0.26	828.65±4.27	2.38±0.30	2.79±0.26
Group-IV	4.50±2.7	4.22±0.25	839.76±1.51	3.67±0.46	4.42±1.23
Group-V	4.09±0.28	3.85±0.24	840.13±0.71	2.53±0.49	3.18±0.10

Table - 5: Effect of aqueous leaf extract of *S. nigrum* pretreatment on serum non-enzymatic antioxidants in normal and experimental rats

Animal Groups	GSH (U/mg protein)	Vitamin-C (µmol/l)
Group-I	50.22±0.64	10.93±0.74
Group-II	20.67±0.37	7.07±0.55
Group-III	41.48±0.35	10.98±1.13
Group-IV	40.10±0.87	13.13±0.16
Group-V	57.79±1.69	11.52±0.12

Table - 6: Effect of aqueous leaf extract of *S. nigrum* pretreatment on phosphorous and magnesium, sodium and chloride, potassium and calcium normal and experimental rats

Animal Groups	Phosphorus (mmol/l)	Magnesium (mmol/l)	Sodium (mmol/l)	Chloride (mmol/l)	Potassium (mmol/l)	Calcium (mmol/l)
Group-I	3.61 ± 0.01	1.54 ± 0.01	142.4±0.25	96.3±0.3	6.3±0.1	6.63±0.01
Group-II	4.70 ± 0.01	2.94 ± 0.01	104.5±0.29	76.3±0.1	3.4±0.01	4.92±0.01
Group-III	3.90 ± 0.01	1.86 ± 0.03	132.4±0.15	89.5±0.35	4.8±0.02	5.93±0.02
Group-IV	3.83 ± 0.02	1.91 ± 0.02	138.2±0.1	92.3±0.11	5.2±0.01	6.32±0.01
Group-V	3.72 ± 0.01	1.57 ± 0.01	135.4±0.21	88.4±0.32	6.93±0.03	6.27±0.02

The results in Table 2 show that there was a significant reduction in Hb level in group II rats when compared to normal rats. AmB induces the gastro intestinal bleeding which leads to excessive loss of erythrocytes, which reduces the Hb level in the blood (Swanson and Cook 1977). AmB is highly hemolytic and decreases the level of Hb and PCV (Yu et al. 1998). It may be due to the presence of active constituents present in *S. nigrum* stimulates the maturation and development of RBC which in turn increases the level of Hb and PCV. The result also shows that there was increase in the platelet count in *S.nigrum* pretreated nephrotoxic rats (group III and group IV) when compared to group I rats. It may be due to the active constituents present in the aqueous extract of *S. nigrum* increases the platelet level in nephrotoxic rats.

AmB induced nephrotoxicity increase the serum urea and BUN level which may be due to damage in proximal tubular epithelial cells which affect the tubular secretion of urea, which will elevates the serum urea level (Nenad et al. 2008). Drug induced nephrotoxicities are associated with marked elevation in BUN (Verpooten et al. 1998). It rises with renal failure, increased protein breakdown and fluid volume depletion. If kidney in not able to remove urea from the blood normally, the BUN rises. Increase in BUN may be due to increased catabolic state of the rats due to the prolonged anorexia associated with AmB induced nephrotoxicity (Adejuwon and Adokiye, 2008). Nephroprotective activity of the plant extract may be due to the active principles contained in *S. nigrum* which possess potent antioxidant

activity (Annie et al. 2005). Table 3 shows that there was a significant (P<0.05) increase in the serum creatinine level in group II AmB induced nephrotoxic rats when compared to group I normal rats. Serum creatinine is an excellent indicator of renal failure. When kidney function was not normal, creatinine levels in the blood rise because it is not adequately excreted by the kidney. Serum creatinine level increased in gentamicin induced nephrotoxic rats (Nenad et al. 2008). Bioactive constituents such as flavonoids, alkaloids etc., present in the aqueous extract of *S. nigrum* prevents the nephrotoxic effect induced by AmB (Ayoola et al. 2006).

Table 3 showed that there was a decrease in the total protein and albumin level in AmB induced nephrotoxic rat. It may be due to AmB stimulates the breakdown of proteins which will decreases the total protein and albumin level (Bennett 1991; Berkersy et al. 2000; Eriksson et al. 2001; Ceylan et al. 2003). There is elevation of total protein and albumin level in *S. nigrum* pretreated nephrotoxic rats (group III & IV) when compared to group II nephrotoxic rats. It may be due to the presence of active constituents such as flavonoids and alkaloids, which may prevent the excessive breakdown of protein.

The results in table 4 shows that there was decrease enzymatic antioxidant activity in kidney tissue in AmB induced nephrotoxic group II rats when compared to group I rats. According to Kumar et al. (2003), AmB induces the peroxide formation, to neutralize its deleterious effect; GPx is involved and there by its level is reduced in the present study (Kumar et al. 2003). AmB induced rats significantly reducing GR, GST, CAT and SOD activity, which may be due to AmB, may induce oxidative stress. To compact the excessive oxidative stress, more enzymatic antioxidant is utilized and leads to number of deleterious reactions. In the present study, there was elevation of these enzymatic antioxidants in *S. nigrum* pretreated nephrotoxic rats. The observed restoration of enzymatic antioxidants may be due to direct stimulatory effect of *S. nigrum* on their activities.

Reduction of non-enzymatic antioxidants (Table 5) may be due to AmB induced the generation of oxygen derived free radicals, which results in lipoperoxidation, protein thiols oxidation, mitochondrial endoplasmic reticulum injury, altered calcium homeostasis and irreversible DNA damage. Acetaminophen induced nephrotoxic rats showed the depletion of glutathione (Schnellman, 2001). *S. nigrum* contains high concentration of bioactive principles such as glycosides, flavonoids, alkaloids and tannins. Flavonoids present in the plant extract prevent the xenobiotic induced nephrotoxicity in experimental animal models which may be due to their potent antioxidant (or) free radical scavenging effects (Devipriya and Shyamaladevim, 1999; Annie et al. 2005).

Table 6 shows that there was elevation in the level of phosphorous and magnesium in AmB induced nephrotoxic group I rats when compared to group II rats. Gentamicin decreases the renal blood flow and glomerular filtration rate with rise in renal vascular resistance which increases the tubular permeability and leads to electrolyte leakage into extra cellular fluid (Bennett 1991). Owing to nephrotoxicity, there may be failure in renal homeostasis which elevates the magnesium level in serum. In the present study the results shows that there was reduction in the level of phosphorous and magnesium in aqueous extract of *S.nigrum* pretreated nephrotoxic rats. The result shows that there was a significant reduction in the level of serum sodium and chloride in group II rats when compared to group I rats. When tubular damage occurs, reabsorption of water and chloride is impaired. So that, poly-urea is present and it is accompanied by the loss of sodium chloride which may lead to lowered plasma chloride and serum sodium (Harold, 1988). In the present study, we have also found the similar result. Electrolyte imbalance may be due to gastrointestinal side effects such as vomiting and diarrhoea. AmB induced nephrotoxicity shows the symptoms of vomiting and diarrhoea which leads to excessive loss of sodium and chloride (Ceylan et al. 2003).

AmB induced nephrotoxicity is known to be manifested by vasopressin resistant poly-urea and hypokalemia. Due to excessive urination there may be excessive loss of potassium and calcium, which ultimately decreases the serum potassium and calcium level. Due to binding of AmB to cell membrane, renal vascular

resistance and tubular permeability increases which leads to the formation of transmembrane pores and leakage of electrolytes (Bolard, 1986; Cheng et al. 1982). AmB induces hypocalemia which is due to the gastro intestinal side effects of the drug such as vomiting and diarrhoea (Bennett 1991; Eriksson et al. 2001). It may be due to the bioactive constituents present in *S. nigrum* prevents the damage of the renal tubular epithelial cells inhibits the electrolyte leakage which elevates potassium and calcium level.

Conclusion

Amphotericin B is the polyene antifungal drug often used intravenously for systemic fungal infections. Nephrotoxicity is the major side effect of AmB and can be severe and irreversible. Nowadays, many medicinal plants are used for the treatment of nephrotoxicity. Phytochemicals in various extracts in *S. nigrum* was screened by standard methods. The result shows that the plant contains phytochemicals such as alkaloids, flavonoids, steroids, terpenoids, glycosides, saponins, tannins, phenols, thiols, resins and anthroquinones. Aqueous extract of *S. nigrum* possess phytochemical constituents. Pretreatment with aqueous extract of *S. nigrum* prevents the biochemical changes to near normal group which suggest that *S. nigrum* has nephroprotective activity. Further studies need to isolate and purify the bioactive principles present in the leaf extract of *S. nigrum* which is responsible for the nephroprotective activity.

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