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Nephroprotective Effect of Ethanolic Extract of *Strychnos Potatorum* Seeds in Rat Models

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ABSTRACT

The study was carried out to evaluate the nephroprotective activity of ethanolic extract of *Strychnos potatorum seeds* in rats. Thirty healthy animals of Wister strain were assigned to five groups. Six animals were randomly separated as normal control group, treated only with normal saline. Remaining 24 animals were randomly assigned to four experimental groups of 6 animals, each receiving gentamicin intra-peritoneally. Since 2000 mg/kg of ethanolic extract of *Strychnos potatorum* did not produce any toxicity, $1/10^{th}$ of the dose was fixed. The extract was administered in three dose levels of 100, 200, 300 mg/kg body weight. Pharmacological studies were carried out to evaluate the haematological and biochemical parameters, followed by histopathological examination. The alcoholic extract of *Strychnos potatorum* at a dose level of 200mg/kg/body weight was found to normalize the raised blood urea, blood protein and serum creatinine. Investigation of the possible protective effect of *Strychnos potatorum* revealed that 10days administration of 200mg/kg of alcoholic extract along with gentamicin reduced the gentamicin induced renal injury. The study concluded that the seeds of *Strychnos potatorum* possess marked nephroprotective activity and could have a promising role in the treatment of acute renal injury induced by nephrotoxins, especially gentamicin.

Key words: *Strychnos potatorum*, nephroprotective, Wister strain, ethanolic extract, control group, gentamicin.

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INTRODUCTION

Dependence of man and his domesticated animals on plants for the essentials of their existence by way of food, clothing and medicines has been dated back to time immemorial. In the present day, the demand for ayurvedic or herbal drugs is increasing day by day globally [1].

Human beings are exposed to environmental, occupational and xenobiotic challenges due to modern lifestyle. Enormous free radicals are generated during the exposure to such stressful challenges. In addition, the process of metabolism and excretion of the xenobiotics may also generate free radicals. These free radicals bind covalently with the tissue macromolecules leading to cell necrosis. Antioxidants are administered to prevent organ toxicities. Herbs containing antioxidant principles have been reported to be highly effective in preventing or curing nephrotoxic conditions [2]. In the present study, the seeds of *Strychnos potatorum* were selected to assess the nephroprotective potential.

Strychnos potatorum Linn. (Family: Loganiaceae), commonly referred to as clearing nut tree or Nirmali is a medium sized glabrous deciduous tree having a height of 6-18 metres [3]. It is a native of India and found distributed in the deciduous forests of West Bengal, central and south India up to 1,200 metres. It is also found in south tropical African countries such as Malawi, Zambia, Zimbabwe, Botswana, Namibia and in Sri Lanka and Myanmar [4].

The plant has been described as a common tree of medicinal importance in India popularly used to purify water for drinking[5]. The seeds contain no strychnine, but brucine is present. They are used to clarify foul and muddy water. They are useful in conditions of hepatopathy, nephropathy, gonorrhea, leucorrhoea, gastropathy, bronchitis, chronic diarrhea, dysentery, strangury, renal and vesicle calculi, diabetes, burning sensation, dipsia, conjunctivitis, scleritis, ulcers and other eye diseases [6].

According to Ayurveda, the seeds are acrid, alexipharmic, lithotriptic and cure strangury, urinary discharges, head ailments etc [7]. Roots cure all types of leucoderma whereas fruits are useful in eye diseases, thirst, poisoning and hallucinations. The ripe fruit is emetic, diaphoretic, alexiteric, cures inflammation, anaemia, jaundice[3]. According to Unani system of medicine, seeds are bitter, astringent to bowels, aphrodisiac, tonic, diuretic and good for the liver, kidney complaints, gonorrhea, colic etc [8].

Since kidney is involved in the clearance of toxins and xenobiotics, it may be more prone to attack by various challenges. A large number of these agents cause damage to these organs by oxidative stress. Therefore, a study on the nephroprotective activity of ethanolic extract of *Strychnos potatorum* (EESP) seeds in gentamicin induced rat models was performed.

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MATERIALS AND METHODS

PLANT MATERIAL

Plant collection and authentication

The seeds of *Strychnos potatorum* (1kg) were collected from Thirunelveli district of Tamil Nadu in the months of April – May. The plant was identified and authenticated by Prof. P. Jayaraman, Ph.D., Director, Plant Anatomy Research Centre (PARC), Tambaram, Chennai and a voucher specimen (PARC/2009/318) was deposited at the Pharmacognosy institute for further reference.

Extraction of plant material

The coarsely dried powder of seeds of *Strychnos potatorum* was used for the extraction procedure. The coarse powder of shade dried seeds of *Strychnos potatorum* (500g) was extracted with 2000 ml of ethanol by cold maceration method in a narrow mouthed bottle with occasional shaking for four days. It was filtered and solvent was removed by distillation under reduced pressure. The residue was then weighed and yield was recorded. The yield of ethanolic extract of *Strychnos potatorum* seeds was found to be 12.7%.

Preliminary phytochemical screening was conducted which included test for phenol, flavanoids, flavones, alkaloids, lignins, glycosides, tannins, steroids and saponins.

EXPERIMENTAL ANIMALS

Adult rats of both strains (Albino & Wister) of either sex weighing 150-300 gms were obtained from animal house. Clearance to carry out the work was obtained from the Institutional Animal Ethical Committee bearing no. 991/C/06/CPCSEA. The animals were placed in a controlled room, in which temperature was maintained at 25±3°C and 35-60% humidity. Normal rat feed and water was provided at regular intervals. All the animals were housed in polypropylene cages having a measurement of 43×27×15 cm. The animals were acclimatized to laboratory conditions before experimental procedures were started.

Acute oral toxicity studies were conducted using the acute toxic class method. The procedure was followed by using OECD 423 guidelines. The method used defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allowed the substances to be ranked and classified according to the Globally Harmonized System (GHS) for the classification of chemical which causes acute toxicity.

Six female Wister rats weighing between 150-300 gm were used for the study. The starting dose level of ethanolic seed extract of *Strychnos potatorum* was 2000 mg/kg. Dose was administered to the rats which were fasted over night but water was provided ad libitum. Food

was withheld for a further 3-4 hours after per oral (p.o.) administration of drugs and observed for signs of toxicity. Body weight of the rats before and after treatment were noted and any changes in skin, fur, eyes, mucous membranes and respiratory, circulatory and behavior pattern were observed and also signs of salivation, nasal discharge, frequent urination, diarrhea, lethargy, sleep were noted. The onset and signs of toxicity were also noted.

In vivo experimental studies were then conducted by assigning 30 healthy animals of Wister strain to five groups. Six animals were randomly separated as normal control group, treated only with normal saline. Remaining 24 animals were randomly assigned to four experimental groups of 6 animals, each receiving gentamicin intra-peritoneally. Since 2000 mg/kg of ethanolic extract of *Strychnos potatorum* did not produce any toxicity, $1/10^{th}$ of the dose was fixed. The extract was administered in three dose levels of 100, 200, 300 mg/kg body weight. Total exposure of the study was 10 days.

Group 1: Solvent control
Animals received a single (p.o.) dose of normal saline at a dose level of 2 ml/kg/day for 10 days.
Group 2: Negative control
Animals received a single intra peritoneal (i.p.) dose of gentamicin at a dose of 100 mg/kg/day for 10 days.
Group 3: EESP (Dose 1)
Animals received a single (i.p.) dose of gentamicin (100 mg/kg/day). Simultaneously, test extracts were given (p.o.) at the low dose level of 100 mg/kg/day for 10 days.
Group 4: EESP (Dose 2)
Animals received a single (i.p.) dose of gentamicin (100 mg/kg/day). Simultaneously, test extracts were given orally at a medium dose level of 200 mg/kg/day for 10 days.
Group 5: EESP (Dose 3)
Animals received a single (i.p.) dose of gentamicin (100 mg/kg/day). Simultaneously, test extracts were given at a high dose level of 300 mg/kg/day for 10 days.

After treatment, the animals in all groups were sacrificed and the kidneys were quickly removed, decapsulated and divided longitudinally into two equally sized pieces. One piece was placed in formaldehyde solution embedded in paraffin and 5 mm thick sections were cut for histopathological examination. Blood (2 ml) was collected from each animal through retro orbital puncture and placed in a clean test tube and centrifuged. The serum obtained was stored at 0 - 4°c for estimation of parameters such as haematological parameters which included total RBC, WBC and haemoglobin content, biochemical parameters like serum urea, serum creatinine, potassium level, total protein and histopathological studies which involved sectioning the kidney longitudinally in two halves and keeping them in 10% neutral formalin solution. Both kidneys were processed and embedded in paraffin wax and sections were taken using a microtome. The sections were stained with hematoxylin eosin and observed under a computerized light microscope. The above mentioned parameters were analyzed using one-way ANOVA followed by Bonferroni-Holm test.

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RESULTS

Extraction of the plant material *Strychnos potatorum* seeds using ethanol by cold maceration method gave a yield value of 12.7%.

Table 1. Qualitative analysis of ethanolic extract of *Strychnos potatorum*

S.No	Phytochemical screening	EESP
1	Phenol	+
2	Flavanoids	+
3	Alkaloids	+
4	Coumarin	—
5	Glycosides	+
6	Tannins	+
7	Fixed oil &fats	—
8	Lignins	+
9	Steroids	+
10	Proteins & Free amino acids	—
11	Gums&Mucilage	—
12	Saponins	+

(+) indicates presence of constituent, (-) indicates absence

Phytochemical screening of the ethanolic extract of *Strychnos potatorum* revealed that phenols, flavanoids, alkaloids, glycosides, tannins, lignins, steroids and saponins were present in the extract whereas constituents like coumarin, fixed oils and fats, proteins and free amino acids, gums and mucilage were found to be absent (Table 1).

The acute toxicity test conducted revealed that no onset of toxicity or signs of toxicity were prevalent after 72 hours of observation. The body weight of the rats was found to be stable and no signs of reversible or irreversible toxicity were reported.

Table 2. Effect of EESP and gentamicin on the body weight of rats

Group	Drug treatment	Average weight of animal (gms)			
		Day 1	Day 3	Day 6	Day 10
Group 1	Normal saline 2 ml/kg (p.o.)	174.16	176.00	178.83	182.50
Group 2	Gentamicin 100 mg/kg (i.p.)	180.66	177.66	174.00	170.00
Group 3	EESP 100 mg/kg (p.o.) + gentamicin 100mg/kg (i.p)	199.16	198.66	198.16	197.50
Group 4	EESP 200 mg/kg (p.o) + gentamicin 100mg/kg (i.p)	209.16	209.66	211.66	213.00
Group 5	EESP 300 mg/kg (p.o) + gentamicin 100mg/kg (i.p)	209.16	209.66	210.00	210.83

Table 2 indicates the effect of EESP and gentamicin on the body weight of rats after 10 days. Group 1, group 4 and group 5 showed slight increase in body weight after administration

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in EESP and/or gentamicin. Group 2 and 3 showed minute decrease in body weight. Figure 1 portrays a comparative study of the body weights of the rats.

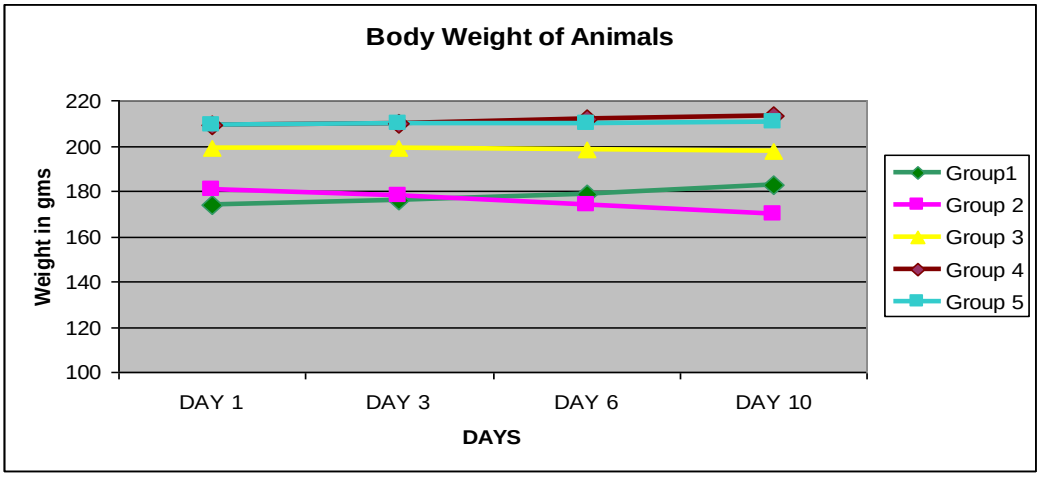


Figure 1. Comparison of body weight of rats

Table 3. Evaluation of haematological parameters

Group	RBC million cells/mm^3	WBC million cells/mm^3	Haemoglobin g%
Group1	7.9 ± 0.086	7850 ± 53.48	12.05 ± 0.531
Group 2	6.25 ± 0.068	8990 ± 82.32	8.183 ± 0.046
Group 3	7.466 ± 0.066	8405.83 ± 70.22	11.166 ± 0.063
Group 4	7.816 ± 0.053	7745 ± 42.01	12.31666667 ± 0.064
Group 5	7.383 ± 0.066	8150 ± 34.15	11.31666667 ± 0.085

Values are given as mean ± SEM of six rats.
Statistical analysis was done by one way analysis of variance (ANOVA).

Table 4. Evaluation of biochemical parameters

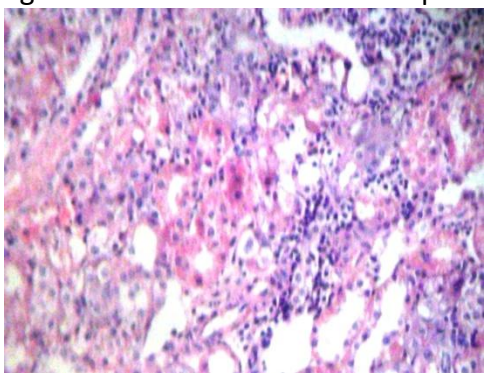
Group	Blood Urea mg/dl	Serum Creatinine mg/dl	Serum Potassium meq/l	Total Protein gm/dl
Group1	41.333 ± 0.501	0.833 ± .020	3.616 ± 0.024	6.15 ± 0.187
Group 2	63.166 ± 0.452	1.75 ± .031	4.8 ± 0.039	4.016 ± 0.231
Group 3	54.666 ± 0.467	1.433 ± .040	4.383 ± 0.038	5.183 ± 0.306
Group 4	42.333 ± 0.512	1.016 ± .019	3.933 ± 0.027	5.733 ± 0.196
Group 5	47.666 ± 0.291	1.033 ± .038	3.866 ± 0.036	5.266 ± 0.250

Values are given as mean ± SEM of six rats.
Statistical analysis was done by one way analysis of variance (ANOVA).

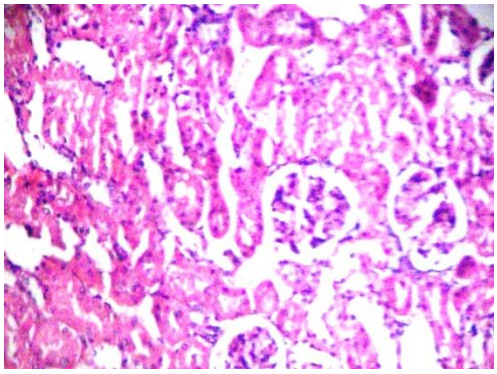
Haematological parameters such as RBC, WBC and haemoglobin count and biochemical parameters like serum urea, serum creatinine, potassium level, total protein were estimated by standard laboratory analytical procedures. The above mentioned parameters were analyzed using one-way ANOVA followed by Bonferroni-Holm test (Table 3 and 4). Comparison between

ISSN: 0975-8585

the groups by Bonferroni-Holm test revealed that the values were found to be statistically significant at P<0.001 when compared to the control.



Group I



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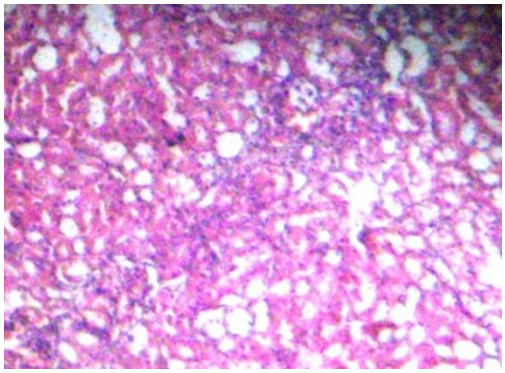

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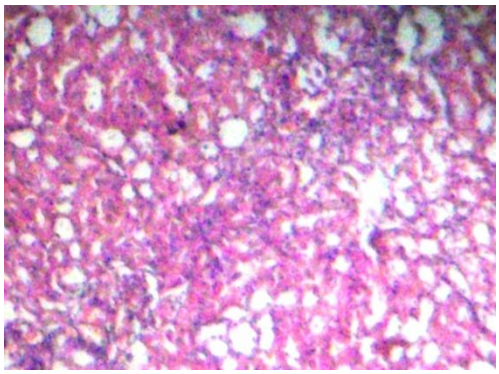
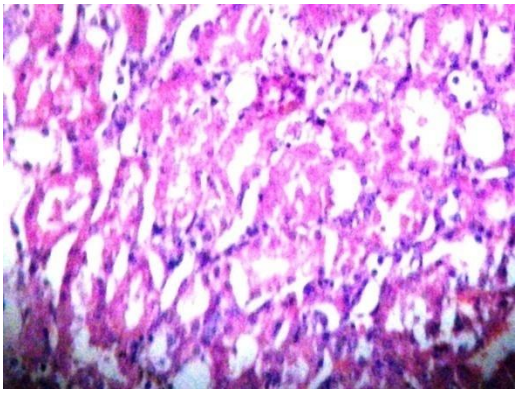


Group IV



Group V

Interpretation of the histopathological slides indicated the following results:

GROUP 1: Solvent control

The section showed normal glomeruli, tubules, few fat vacuoles in interstitium and normal blood vessels.

GROUP 2: Negative control
Showed glomerular congestion, minimal tubular necrosis and interstitial infiltrate (lymphocytes), blood vessels congested. Appearance of numerous violet dots indicated necrosis and cell congestion.
GROUP 3: EESP (dose 1)
Showed normal glomerulus, focal minimal tubular damage, occasional interstial infiltrate, tubular cast and normal blood vessels.
GROUP 4: EESP (dose 2)
Showed normal glomerulus, normal tubular interstitium and normal blood vessels.
GROUP 5: EESP (dose 3)
Showed normal glomerulus, tubules and few fat vacuoles in interstium and normal blood vessels.

DISCUSSION

The present study was undertaken to establish the nephroprotective effect of *Strychnos potatorum*. Single administration of gentamicin daily for 10 days produced a significant increase in blood urea, serum creatinine and reduction in protein level, followed by significant loss of body weight in the experimental animals.

The alcoholic extract of *Strychnos potatorum* at a dose level of 200mg/kg body weight was found to normalize the raised blood urea, blood protein and serum creatinine levels. The animals showed signs of recovery and an increase in the body weight was observed on the final day of observation.

While investigating into possible protective effect of *Strychnos potatorum*, it was observed that 10 days of administration of 200mg/kg of alcoholic extract along with gentamicin reduced the gentamicin induced renal injury.

Histopathological evaluation revealed that only gentamicin treated animals showed tubular necrosis with interstitial inflammatory infiltrate and congestion of blood vessels. Whereas, the gentamicin + EESP treated animals showed normal tubules and blood vessels with no congestion. Ethanolic extract of *Strychnos potatorum* in a dose of 200mg/kg body weight was better in normalizing the renal tissues, when compared to the other two dose levels.

CONCLUSION

The study concluded that the seeds of *Strychnos potatorum* possess marked nephroprotective activity and could have a promising role in the treatment of acute renal injury induced by nephrotoxins, especially gentamicin. The histopathological evaluation revealed that dose 1 has mild action where as 2 & 3 are highly effective against gentamicin induced necrosis. The study evaluated the nephroprotective effect of alcoholic extract of *Strychnos potatorum*

and identified it to posses significant nephroprotective activity. However, further studies are required to identify and isolate the active principle, which is responsible for this effect.

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