Toxicological Investigations on *Strychnos potatorum* Linn Seeds in Experimental Animal Models

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The acute and chronic toxicity studies of aqueous extract (SPE) and seed powder (SPP) of *Strychnos potatorum* (*S. potatorum*) Linn were carried out in Wistar albino mice and rats, respectively. The animals did not show any toxic effects upto the dose of 2000 mg/kg, p.o. According to OECD guidelines — 423 for acute oral toxicity, the LD50 dose of 2000 mg/kg and above is categorized as "unclassified." For chronic toxicity study, 100 and 200 mg/kg, p.o. of SPE and SPP were administered to Wistar rats for 90 days and various parameters like food and water intake, body weight changes, haematological parameters like red blood corpuscles (RBC), white blood corpuscles (WBC), haemoglobin (Hb) and erythrocyte sedimentation rate (ESR), biochemical parameters like blood glucose and urea, serum creatinine, enzyme parameters like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and acid phosphatase (ACP) were studied. There were no significant changes in any of the above parameters of drug treated groups with respect to control group, which explain their nontoxic nature. Further the nontoxic effect of the drugs SPP and SPE were confirmed by histopathological examination of various organs like liver, kidney, spleen and heart. Phytochemical studies of the drugs showed the presence of carbohydrates, alkaloids, steroids/triterpenes, polyphenolics, saponins and polysaccharides in SPP and carbohydrates, steroids, triterpenes, saponins, polyphenolics and polysaccharides in SPE.

Key words —— *Strychnos potatorum*, acute and chronic toxicity, haematological test, biochemical parameter

INTRODUCTION

Strychnos potatorum (*S. potatorum*) Linn (Fam: Loganiaceae) is a moderate sized tree found in southern and central parts of India, Sri Lankha and Burma.[1)]

The seeds are bitter, astringent, refrigerant, demulcent, emetic, diuretic, digestive, stomachic, anthelmintic, aphrodisiac, ophthalmic, appetizer, alexiteric, tonic and water purifier, relieve colic. In ayurvedic system of medicine, the seeds are used in vitiated conditions of kapha and vata, hepatopathy, nephropathy, gonorrhea, leucorrhoea, gastropathy, bronchitis, chronic diahorrea, dysentery, strangury, renal and vesicle calculi, diabetes, burning sensation, dipsia, conjunctivitis, scleritis, ulcers and other eye diseases.[2)] The ripe seeds are used for clearing muddy water. They are reported to be very effective as coagulant aids. The clarification is due to the combined action of colloids and alkaloids in the seeds.[3)]

Although *S. potatorum* is widely used in traditional medicine, there exists a controversy on its toxic effect as expected with another species *Strychnos nuxvomica*. The present study reports the results of acute and chronic toxicity of seed powder (SPP) and studies of aqueous extract (SPE) in animals supplemented with biochemical and histopathological studies.

MATERIALS AND METHODS

Collection of Specimen (*S. potatorum* Linn Seeds) —— The seeds specimen for the study were collected from crude drug market, Chennai and the authenticity of the seed specimen was confirmed by

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Preparation of the Extract ——— The air-dried seeds were coarsely powdered and subjected to hot water extraction for 2 hr at 100°C, it was then filtered and the filtrate was evaporated to dryness. A grey colored semisolid mass was obtained which was dried under vacuum and kept in a dessicator. The percentage yield of the extract (SPE) was 22.5%(w/w) from the starting crude material. The SPP as such was used for the treatment. For the experimental study, both the drugs (SPP and SPE) were triturated with distilled water and administered immediately.

Phytochemical Screening ——— Preliminary phytochemical analysis was carried out as per the methods of Kokate[4] and Trease and Evans.[5]

Animals Used ——— Wistar albino mice (20 ± 5 g) and rats (140 ± 20 g) procured from Tamilnadu University of Veterinary and Animal Sciences (TANUVAS) were used for the study. The animals were kept in polypropylene cages and maintained at a temperature of 22 ± 2°C. They were fed with standard pelleted feed (TANUVAS) and water ad libitum. The study has got the approval from the Institutional Animal Ethical Committee (IAEC) of Committee for the Purpose of Control and Supervision of Experiments on Animals, Govt. of India (CPCSEA).

Acute Toxicity Study ——— Wistar albino mice of either sex were used in this study. Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class).[6] Wistar mice (n = 6) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the drugs SPP and SPE were administered orally at the dose level of 5 mg/kg body weight by gastric intubation and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated as above for further higher doses such as 50, 300 and 2000 mg/kg body weight. The animals were observed for toxic symptoms like behavioral changes, locomotion, convulsions etc. and mortality for 72 hr.

Chronic Toxicity Study ——— The Wistar albino rats of either sex were used for the study and they were divided into 5 groups, each containing 6 animals. Group I served as control, Groups II & III were given SPP at the doses of 100 (SPP-I) and 200 (SPP-II) mg/kg, p.o., respectively. Groups IV & V were given SPE at the doses of 100 (SPE-I) and 200 (SPE -II) mg/kg, p.o., respectively.

The control group received distilled water orally and the remaining groups received the respective drug treatments for 90 days. The gross changes like body weight, food and water intake were recorded at weekly intervals along with the simultaneous observation for toxic symptoms and mortality, if any. Rats were sacrificed at the end of 90 days by excess anesthesia, blood was collected for glucose, urea estimation and hematological studies. Serum was separated and used for the estimation of creatinine. The liver and kidney were isolated and processed for biochemical investigations. Sections of liver, kidney, heart and spleen were dissected out and kept in 10% formalin for histopathological studies.

Haematological Tests ——— The blood was collected into test tubes containing EDTA. Erythrocyte count was estimated by the Hemocytometer method of Ghai.[7] Total leukocyte count was estimated by the Hemocytometer method of John.[8] Haemoglobin was estimated by the method of Ghai.[7] ESR was estimated by the method of Wintrobe and Landberg.[9]

Biochemical Analysis ———

Estimation of Glucose, Urea and Creatinine: Glucose was estimated by the method of Sasaki *et al*.[10] blood urea nitrogen was estimated by the method of Natelson *et al*.[11] and creatinine was estimated by the method of Slot.[12]

Enzyme Parameters: Aspartate aminotransferase (AST) (2.6.1.1) was estimated by the method of King.[13] Alanine aminotransferase (ALT) (2.6.1.2) was assayed by the method of King.[13] Alkaline phosphatase (ALP) (3.1.3.1) was assayed by the method of King.[14] Acid phosphatase (ACP) (3.1.3.2) was assayed by the method of King.[14]

Statistical Analysis: The data represents mean ± S.E.M. Results were analysed statistically using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison.

The minimum level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Acute Toxicity Study

Both drugs SPP and SPE did not produce any signs of toxicity or mortality upto the dose level of

2000 mg/kg body weight orally in mice. According to OECD guidelines — 423 for acute oral toxicity[6] the LD50 dose of 2000 mg/kg and above is categorized as unclassified. Hence the drugs SPP and SPE were considered to be safe upto the dose level of 2000 mg/kg, p.o.

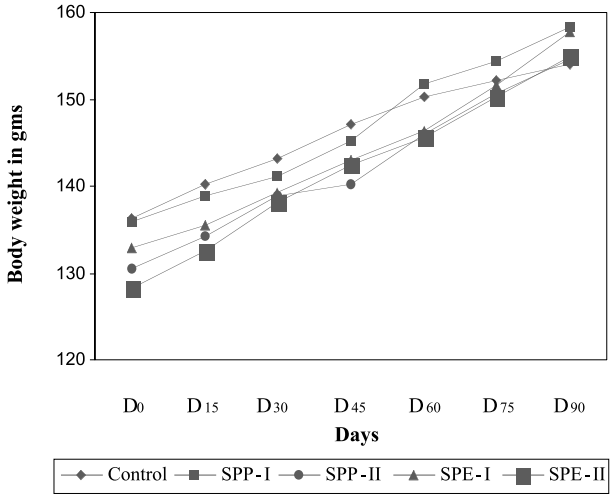

Fig. 1. Effect of SPP and SPE on Body Weight Changes during 90 days Treatment in Chronic Toxicity Study

No significant changes were seen in drug treated groups compared with control.

Chronic Toxicity Study

In chronic toxicity study, both SPP and SPE at the dose of 100 and 200 mg/kg, p.o. did not produce any significant changes in body weight from day 0 (D_0) to day 90 (D_{90}) when compared with the control group (Fig. 1). Generally, changes in body weight have been used to assess the course of the disease and the response to therapy of drugs,[15] also they indicate the adverse effects of drugs.[16] Thus the present result suggests that SPP and SPE at 100 and 200 mg/kg, p.o. are non toxic in rats.

The effects of SPP and SPE at 100 and 200 mg/kg, p.o. on hematological parameters in rats are given in Table 1. The drugs did not show any significant changes in red blood corpuscles (RBC), white blood corpuscles (WBC), haemoglobin (Hb) and erythrocyte sedimentation rate (ESR), when compared with the control group and also the values are within normal range.[17]

Table 2 depicts the levels of blood glucose, urea and serum creatinine in rats administered SPP and SPE (100 and 200 mg/kg, p.o.) for 90 days. There were no significant changes in any of the above parameters in drug treated groups when compared with the control group. The normal range of blood glucose in all the groups similar to control shows the normoglycemic activity of the drugs (SPP and SPE). Determination of blood urea and serum creatinine

Table 1. Effect of Chronic Treatment (90 days) of SPP and SPE on Haematological Parameters

Groups	RBC (millions/mm^3)	WBC (thousands/mm^3)	ESR (mm/hr)	Hb (g/dl)
Control	5.78 ± 0.20	7.82 ± 0.15	1.65 ± 0.08	13.68 ± 0.26
SPP-I	5.71 ± 0.17	8.03 ± 0.10	1.83 ± 0.09	13.21 ± 0.34
SPP-II	5.86 ± 0.23	8.11 ± 0.25	1.96 ± 0.28	14.11 ± 0.63
SPE-I	5.84 ± 0.20	7.90 ± 0.35	1.61 ± 0.25	14.15 ± 0.33
SPE-II	6.11 ± 0.22	7.94 ± 0.24	1.74 ± 0.12	13.73 ± 0.20

Data represents mean ± SEM of 6 animals (One way ANOVA). No significant changes were seen between drug treated and control groups.

Table 2. Effect of Chronic Treatment (90 days) of SPP and SPE on Blood Glucose and Urea, Serum Creatinine in Experimental Rats

Groups	Glucose (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control	97.50 ± 1.05	25.16 ± 0.87	1.47 ± 0.05
SPP-I	97.00 ± 0.92	23.00 ± 0.84	1.24 ± 0.06
SPP-II	97.40 ± 1.21	24.83 ± 0.86	1.33 ± 0.05
SPE-I	99.20 ± 1.41	24.16 ± 0.89	1.17 ± 0.04
SPE-II	98.30 ± 0.88	24.00 ± 0.88	1.34 ± 0.03

Data represents mean ± SEM of 6 animals (One way ANOVA). No significant changes were seen between drug treated and control groups.

Table 3. Effect of Chronic Treatment (90 days) of SPP and SPE on Liver Marker Enzymes

Groups	ALT	AST	ALP	ACP
Control	2.97 ± 0.46	2.51 ± 0.32	2.71 ± 0.41	1.17 ± 0.33
SPP-I	2.98 ± 0.32	2.48 ± 0.39	2.67 ± 0.45	1.22 ± 0.45
SPP-II	2.92 ± 0.42	2.63 ± 0.42	2.82 ± 0.34	1.16 ± 0.39
SPE-I	3.07 ± 0.28	2.39 ± 0.27	2.79 ± 0.21	1.14 ± 0.47
SPE-II	3.10 ± 0.33	2.52 ± 0.33	2.76 ± 0.45	1.24 ± 0.21

Data represents mean ± SEM of 6 animals (One way ANOVA). No significant changes were seen between drug treated and control groups. ALT & AST (μmol $\times 10^{-3}$ pyruvate formed/min/mg protein). ALP & ACP (μmol $\times 10^{-3}$ phenol liberated/min/mg protein).

Table 4. Effect of Chronic Treatment (90 days) of SPP and SPE on Kidney Marker Enzymes

Groups	ALT	AST	ALP	ACP
Control	3.06 ± 0.21	2.46 ± 0.33	1.56 ± 0.45	1.84 ± 0.22
SPP-I	3.15 ± 0.44	2.51 ± 0.42	1.48 ± 0.32	1.84 ± 0.48
SPP-II	3.00 ± 0.33	2.41 ± 0.26	1.45 ± 0.27	1.90 ± 0.19
SPE-I	2.99 ± 0.21	2.49 ± 0.29	1.52 ± 0.59	1.78 ± 0.45
SPE-II	3.02 ± 0.19	2.50 ± 0.27	1.55 ± 0.33	1.92 ± 0.34

Data represents mean ± SEM of 6 animals (One way ANOVA). No significant changes were seen between drug treated and control groups. ALT & AST (μmol $\times 10^{-3}$ pyruvate formed/min/mg protein). ALP & ACP (μmol $\times 10^{-3}$ phenol liberated/min/mg protein).

showed that the drugs SPP and SPE did not produce any renal dysfunction, as increase in the above parameters reveals the impaired renal function or acute renal failure.[18)]

Tables 3 and 4 indicate the levels of transaminases (ALT & AST) and phosphatases (ALP & ACP) in liver and kidney tissues of rats treated with SPP and SPE for 90 days. The drugs did not alter the enzyme levels when compared with the control. ALT and AST are good indices of liver function.[19)] Hence, it is reasonable to deduce that SPP and SPE did not induce any pathological damage to the liver and kidney. This was further confirmed by the histopathological assessment of these organs.

Histopathology

The histopathological studies of the major vital organs like liver, kidney, spleen and heart recovered from the control and treated groups showed normal architecture suggesting no detrimental changes and morphological disturbances were caused by the daily oral administration of both SPP and SPE for 90 days.

The results of present investigation show the nontoxic nature of the drugs (SPP and SPE) and thus the drugs can be used for long-term treatment.

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