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Anti-inflammatory Effect of *Strychnos potatorum* Seeds on Acute and Subacute Inflammation in Experimental Rat Models

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Abstract

The anti-inflammatory effect of seed powder [SPP I (100 mg/kg) and II (200 mg/kg) p.o.] and aqueous extract [SPE I (100 mg/kg) and II (200 mg/kg) p.o.] of *Strychnos potatorum* Linn (Loganiaceae) seeds was studied in carrageenin-induced hind paw edema and cotton pellet granuloma models. In carrageenin-induced rat paw edema model, both SPP (I and II) and SPE (I and II) exhibited mild inhibition at 1 h and maximum inhibition at 2.5 h in a dose-dependent manner. In the cotton pellet granuloma model, both wet and dry weights of the cotton pellets were significantly (p < 0.001) inhibited by SPP and SPE at both dose levels, whereas a maximum (p < 0.001) inhibition of wet and dry weights was found at 200 mg/kg, p.o. Both SPP and SPE were found to normalize the increased alkaline, acid phosphatases, and lipid peroxide levels indicating their membrane stabilization and free radical scavenging properties, respectively. In conclusion, both SPP (I and II) and SPE (I and II) exhibited anti-inflammatory activity in acute and subacute inflammatory models, whereas SPE-II was found to possess maximum activity and its effect was also comparable with the standard drug diclofenac sodium (5 mg/kg, p.o.).

Keywords: Carrageenin-induced paw edema, cotton pellet granuloma, diclofenac sodium, *Strychnos potatorum*.

Introduction

*Strychnos potatorum* Linn (Loganiaceae) is a medium-sized deciduous tree found in southern and central parts of India, Sri Lanka, and Burma (Kirtikar & Basu, 1933). The seeds are bitter and used as an astringent, demulcent, emetic, diuretic, stomachic, and also used to purify water. They are used in vitiated conditions of *kapha* and *vata*, hepatopathy, nephropathy, gonorrhea, leucorrhea, gastropathy, bronchitis, chronic diarrhea, dysentery, strangury, renal and vesicle calculi, diarrhoea, for burning sensation, dysipsia, conjunctivitis, scleritis, ulcers and other eye diseases (Asima & Satyesh, 2001).

Phytochemical investigations on the seeds revealed the presence of diaboline (major alkaloid) and its acetate (Harkishan Singh et al., 1975) brucine, loganin, mannose, sucrose, and arachidonic, lignoceric, linoleic, oleic, palmitic, and stearic acids (Singh & Bajpai, 1975), β-sitosterol, stigmasterol, oleic acid and its 3β-acetate, saponins containing oleanic acid, galactose, and mannose (Singh & Dhar, 1977), triterpenes, sterols (Harkishan Singh & Kapoor, 1975), and mannogalactans (Corsaro et al., 1995). Although the seeds contain a number of phytochemicals, they have not been evaluated for their pharmacological activities in detail. Hence, this work was carried out to find the anti-inflammatory activity of the seed powder (SPP) and aqueous extract (SPE) of *Strychnos potatorum* seeds against acute and subacute inflammatory models in rats.

Materials and Methods

Animals

Wistar albino mice (20–25 g) and rats (140 ± 20 g) of either sex procured from TANUVAS (Tamilnadu University of Veterinary and Animal Sciences) were used.
for the study. The animals were kept in polypropylene cages at a temperature of 22 ± 2°C. They were fed with standard pelleted feed (TANUVAS) and water ad libitum. The study was approved by the institutional animal ethical committee (IAEC).

**Plant material**

The seed specimen used for the study was collected from a crude drug market (Chennai, India). The identity of the seed specimen was confirmed by Dr. S. Jayaraman, Botanist, Plant Anatomy Research Centre (Chennai, Tamilnadu, India). A voucher specimen (SP-02/02) has been deposited in the Department of Pharmacology & Environmental Toxicology, University of Madras, Chennai.

**Preparation of the extract**

The air-dried seeds were coarsely powdered and subjected to hot water extraction for 2 h at 100°C; it was then filtered through muslin cloth and the filtrate was evaporated to dryness. A gray-colored semisolid mass was obtained, which was dried under vacuum and kept in a desiccator. The percentage yield of the extract (SPE) was 22.5% w/w from the starting crude material. The seed powder (SPP) used for the study was prepared by grinding the dried seeds in a blender. For the experiment, both SPP and SPE were triturated with distilled water and administered immediately by oral route.

**Phytochemical analysis**

SPP and SPE were subjected to phytochemical screening through qualitative chemical analysis and HPTLC finger printing.

**Acute toxicity study**

Wistar albino mice of either sex weighing 20–25 g selected by random sampling technique were used in this study. Acute oral toxicity was performed as per OECD-423 guidelines (acute toxic class) (Ecobichon, 1997). The animals were fasted overnight, provided only water, after which the drugs SPP and SPE were administered to the respective groups orally at the dose level of 5 mg/kg body weight by gastric intubation and the groups observed for 14 days. If mortality was observed in 2 of 3 animals, then the dose administered was assigned as a toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such as 30, 300, and 2000 mg/kg body weight. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions, and mortality for 72h.

**Anti-inflammatory activity**

The animals were divided into six groups each containing six rats. Group I served as vehicle control, group II received diclofenac sodium (5 mg/kg, p.o.) as standard drug. Groups III–VI received SPP [I (100 mg/kg) and II (200 mg/kg)] and SPE [I (100 mg/kg) and II (200 mg/kg)] p.o., respectively.

**Carrageenin-induced rat paw edema**

The acute anti-inflammatory activity was evaluated by carrageenin-induced rat paw edema according to the method of Winter et al. (1962). Edema was induced in rats by injecting 0.1 mL of a 1% solution of carrageenin in saline into the plantar aponeurosis of the left hind paw. The drugs were given orally 1 h prior to the injection of carrageenin. The volumes of edema of the injected and contralateral paws were measured at +1, 2.5, and 5.5 h after induction of inflammation using a mercury displacement plethysmometer, and the percentage inhibition of edema was calculated.

**Cotton pellet granuloma**

Under light ether anaesthesia, sterile cotton pellets (weighing 10 ± 2 mg) were implanted subcutaneously along the flanks of axillae and groins of Wistar albino rats (Swingle & Shideman, 1972). The SPP (I and II), SPE (I and II), and diclofenac sodium (5 mg/kg) were dissolved in distilled water and administered orally to rats every day for a period of 7 days. On day +8, the rats were sacrificed by cervical decapitation and cotton pellets were removed surgically, freed from extraneous tissue, and weighed immediately for wet weight. One half of the pellets were dried at 60°C until a constant dry weight was obtained.

**Biochemical parameters**

Blood was collected and the serum was separated in plain tubes. The liver tissue was perfused with 0.86% cold saline to completely remove all the red blood cells. It was placed in 10% w/v ice-cold 0.1 M phosphate buffer (pH 7.4), cut into small pieces, and the required quantity was weighed and homogenized using a Teflon homogenizer. Serum and liver homogenates were used for the estimation of different biochemical parameters. Lipid peroxide (LPO) content in the liver homogenate and in the exudates collected from the remaining pellets was assayed by thio- barbituric acid procedure of Ohkawa et al. (1979), and the percentage inhibition of lipid peroxidation (LPO) was calculated. Alkaline and acid phosphatases (ALP and ACP) were assayed in serum and liver using disodium phenyl phosphate as substrate (King, 1965a). The enzyme activities are expressed as enzyme units/mg of protein.
**Statistical analysis**

The data represents mean ± SEM. Results were analyzed statistically using one-way ANOVA followed by Tukey’s multiple comparison. The minimum level of significance was set at $p < 0.05$.

**Results**

Phytochemical analysis of drugs SPP and SPE revealed the presence of steroids, triterpenes, alkaloids, saponins, polyphenolics, reducing sugars, and polysaccharides. The HPTLC screening further supported the presence of steroids, triterpenes, saponins, alkaloids, and polysaccharides.

**Acute toxicity study**

Neither SPP nor SPE produced any toxic symptoms or mortality up to the dose level of 2000 mg/kg body weight orally in rats, and hence the drugs were considered to be safe for further pharmacological screening. According to OECD-423 guidelines for acute oral toxicity, the LD$_{50}$ dose of 2000 mg/kg and above is categorized as unclassified.

**Anti-inflammatory activity**

*Carrageenin-induced rat paw edema*

Pretreatment of animals with SPP (I and II) and SPE (I and II) resulted in a significant and dose-related inhibition of carrageenin-evoked hind paw edema. The standard drug, diclofenac sodium, showed significant edema inhibition in all the phases, whereas SPP (I and II) and SPE (I and II) showed mild inhibition ($p < 0.05$, $p < 0.01$), respectively at 1 h, maximum inhibition ($p < 0.001$) at 2.5 h, and again the edema inhibition decreased at 5.5 h. Among all the groups, SPE-II exhibited greater anti-inflammatory activity (56.84%) at 2.5 h, which was comparable with the standard drug (66.31%) (Fig. 1).

*Cotton pellet granuloma*

It can be noted from Figure 2, that all the drugs showed significant ($p < 0.001$) activity in inhibiting wet weight of granuloma, whereas SPE-II possessed greater activity (44.71%) than all other tested groups, but less than the standard drug, diclofenac sodium (50.7%). On dry weight basis, SPE-II (52.16%) showed greater activity than all other drugs and the standard (44.58%) (Fig. 2).

The effects of SPP and SPE on various biochemical parameters in the exudates, serum, and liver of rats exposed to cotton pellet are summarized in Table 1. The levels of lipid peroxides (liver and exudates) as well as phosphatase enzymes (serum and liver) in cotton

**Discussion**

Accumulation of edema fluid as a function of time after the subplantar injection of the irritant (carrageenin) in rats is biphasic (Vinegar et al., 1969). Histamine and
serotonin are usually responsible for eliciting the immediate response of inflammation in rats (first phase), whereas the kinins and prostaglandins (PG) mediate the more prolonged delayed onset responses (second phase) (Vane & Botting, 1987). From the results obtained, it can be inferred that the inhibitory effects of the drugs SPP and SPE on carrageenin-induced inflammation in rats could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of PG synthesis. The decrease in edema inhibition at 5.5 h may be due to increased generation of leukotrienes at that stage caused by the inhibition of PG synthesis in the second phase because inhibition of PG synthesis diverts the reaction toward increase in leukotriene synthesis (Mayes, 1996).

Three phases of the inflammatory response to a subcutaneously implanted cotton pellet in the rats have been described: (1) a transudative phase, defined as the increase in wet weight of the pellet that occurred during the first 3 h; (2) an exudative phase, occurring between 3 and 72 h after implanting the pellet; (3) a proliferative phase, measured as the increase in dry weight of the granuloma that occurs between 3 and 6 days after implantation (Swingle & Shideman, 1972). The results indicate that both SPP (I and II) and SPE (I and II) exhibited significant (p < 0.001) antitransudative and antiproliferative effects by inhibiting both the wet and dry weights of cotton pellets when compared with the control. The antitranusclative effects of the drugs were less than the standard diclofenac sodium, whereas the antiproliferative effect of SPE-II was greater than that of the standard drug.

The role of lipid peroxidation in the development of inflammation is well documented (Bonta et al., 1980). All the major biomolecules like lipids, proteins, and nucleic acids may be attacked by free radicals, but lipids are probably the most susceptible (Cheeseman & Slater, 1992). The oxidative destruction of lipids (lipid peroxidation) is a destructive, self-perpetuating chain reaction, releasing malondialdehyde (MDA) as the end product (Cheeseman, 1993). The increase in lipid peroxide formation in the liver and exudates of cotton pellet granuloma–induced rats indicates the inflammation at different sites might have an adverse effect probably due to the release of autacoids (Karunakar et al., 1997). In the current study, SPP and SPE have been found to reduce the lipid peroxide level in liver tissue and exudates of experimental animals. This observation indicates that SPP and SPE may function as a free radical scavenger (Table 1).

Lysosomal enzyme activities in inflammatory exudates serve as a good marker to assess the intensity of inflammation in experimental groups. Hydrolytic enzymes are released by the rupture of the lysosomal membrane, which in turn indicates the synthesis of inflammatory mediators such as thromboxanes, prostaglandins, and leukotrienes. Drugs capable of stabilizing the lysosomal membrane can reduce inflammation (Agha & Gad, 1995). In the current study, the level of serum and liver phosphatases was raised during inflammation and it was normalized by SPP and SPE treatment (Table 1). This reversal effect of the test drugs (SPP and SPE) on decrease in serum and liver phosphatases activity indicates that the test drugs may have an effect on membrane stabilization.

In preliminary phytochemical and HPTLC screening, both SPP and SPE revealed the presence of steroids, triterpenoids, saponins, and polyphenols. The anti-inflammatory activities of these phytochemicals have been well documented in the literature (Parmar & Ghosh, 1978; Garcia et al., 1999; Li et al., 2002; Wei et al., 2004). Hence, the presence of these phytochemicals might be responsible for the anti-inflammatory activity of the drugs SPP and SPE.

References


