



EVALUTION OF ANTI-INFLAMMATORY ACTIVITY *SCINDAPSUS OFFICINALIS* FRUIT IN CARRAGEENAN-INDUCED PAW OEDEMA RATS

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Article Received on
06 June 2018,

Revised on 26 June 2018,
Accepted on 16 July 2018

DOI: 10.20959/wjpps20188-12073

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ABSTRACT

The plant *Scindapsus officinalis* (Roxb.) Schott fruits are widely used in many parts of India for the treatment of various diseases and ailments. It is one of the plants used in Indian system of medicine which belongs to family aracea. The plant is growing in tropical parts of India. The fruit of *Scindapsus officinalis* is known as Gajpeepal in ayurveda. Gajpeepal consists of dried, transversely cut pieces of mature female spadix of *Scindapsus officinalis* (Fam. Araceae).

Material: Group I-Control, animals were treated with 10% Tween-80 *p.o*, Group II--Standard group, animals were treated with 10mg/kg Indomethacin, Group III-Animals were treated with 30mg/kg b wt. *p.o* of isolated piperine from *Sindapsus officinalis* fruits. Group IV- Animals were treated with 60mg/kg b wt. *p.o* of isolated piperine from

Sindapsus officinalis fruit. **Results & Discussion:** Animals treated with standard drug like piperine (10mg/kg) produces highly significant paw edema volume when compared to control animals. Whereas, high dose of 400 mg/kg isolation of piperine from *scindapsus officinalis* fruit produces highly significant decreased paw edema volume which is similar to the response of Piperine treatment. When compared to control animals. In case of low dose 200 mg/kg isolation of piperine from *scindapsus officinalis* fruit produces significant decreased paw edema volume when compare to control animals.

KEYWORDS: *Scindapsus officinalis*, Gajpeepal, anti inflammatory.

It has the significant antioxidant property due to presence of flavonoids and phenolic compound and have ability of cytoprotection due to antioxidant property we highlight the Anti inflammatory properties of fruit of *Scindapsus*.

INTRODUCTION

Scindapsus officinalis (Roxb.) Schott. is one of the plant used in Indian system of medicine which belongs to family Araceae. The plant of *Scindapsus officinalis* is a large, stout, epiphytic and perennial climber with adventitious aerial roots growing on trees and rocks (Figure 1).^[1,2] The plant is growing in tropical part of India. It is common in the Midnapore district of west Bengal and cultivated vegetatively for its fruit, which is cut into transverse pieces, dried and used medicinally.^[4,6] Fruit (Figure 2) is very important part of the plant and accepted as raw drug of known properties in both Ayurvedic and Unani system of medicine. The fruit is reported to be useful as a diaphoretic, carminative stimulant, anthelmintic aphrodisiac, galactagogue, appetizer and also useful in the form of decoction in diarrhea, asthma and other affections supposed to be caused by Kafa.^[7,10] Anatomical/histological practice playing a unique role in the more detailed examination of crude drugs and can be used to confirm the structural features of the crude drugs. Quantitative microscopy and linear measurements are the other important aspects of the histological method.^[11] The histological approach to study plants and plants parts is helpful in the searching of specific microscopical characters and even some times it is helpful in the differentiation between two species of same genus. Based on this fact and Since no complete anatomical data related to fruit is available so far. It has the significant antioxidant property due to presence of flavonoids and phenolic compound and have ability of cytoprotection due to antioxidant property we highlight the Anti inflammatory properties of fruit of *Scindapsus*.



Figure 1: Plant of *Scindapsus officinalis* with fruit.



Figure 2: Shade dried fruit of *Scindapsus officinalis*.

MATERIALS AND METHODS

Isolation of Piperine from *scindapsus officinalis* fruit

Place 50gm of grinded *scindapsus officinalis* fruits in 250ml round bottom flask add 500 ml of 95% ethanol 5 boiling chips and reflux for 2hours. Filter the mixture by suction filtration and then concentrate the filtrate to a volume of 10-15ml by simple distillation. To 50ml of a 10% solution of potassium hydroxide add the concentrated above alcoholic extract. The resulting solution was heated and add water drop wise. A yellow precipitate was formed. Add water until no more solid appears to form and then allow the mixture to stand at least over night. Collect the solid by suction filtration and recrystallize it by acetone.^[12]



Fig. 3: Isolated piperine from *scindapsus officinalis* fruit.

Photochemical investigation

Piperene was tested for Carbohydrates, proteins, amino acids, Alkaloids, Glycosides, Flavonoids, Phytosterols, Fats and oils, Phenolics and tannins and Volatile oils.

Determination Percentage Yield

Isolation of piperine by using 95% ethanol by reflux method.

Determination of Loss on Drying or Moisture Content

Loss on drying is the loss in weight in %w/w, determined as per the following method. Weighed 1gm of isolated piperine into a weighed flat and thin porcelain dish. Dry in the oven at 100⁰C or 105⁰C. Cool in a desiccators and watch the loss in weight and is usually recorded as moisture. The percentage of loss on drying was calculated with reference to the air dried drug.

Melting Point Determination

Melting point of piperine was determined by open capillary method and was found to be 140⁰C – 300⁰C. This results are tabulated in.^[13]

Thin Layer Chromatography

Preparation of plates

The adsorbent used for thin layer chromatography was silica gel G. About 25 gm of silica gel G was taken in a glass mortar and about 35ml distilled water was added to it. The mixture was stirred with glass rod until it become homogeneous and allowed to well for 15min. 15ml of distilled water was added to it with stirring. The suspension was then transferred to a 150 ml flask fitted with a stopper and was shaken vigorously for about 2 minutes. This suspension was uniformly spreaded immediately on thin layer chromatographic plates.

Drying and storage of plates

The freshly coated plates were then air dried and stocked in a drying rack and were heated in a oven for 30 min at 110⁰C. Activated plates were kept in a desiccators, till required for further use.

Preparation of test sample

10 mg of test sample of piperine was dissolved in 5ml of 95% of ethanol. Drop was applied as a spot.

Application of the sample

The sample was applied in the form of spot. The spot was applied with the help of fine capillaries. Spot was marked on the top of the plate for their identification.

Developing solvent system

A number of developing solvent systems were tried, but the satisfactory resolution was obtained in the solvent systems .After development of plates, they were air-dried and number

of spots were noted and R_f values were calculated. Spots were visualized by dragendroffs reagent, UV chamber and iodine vapours detecting agents.^[14]

Number of spots were noted and R_f values were calculated using the formula,

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Pharmacological Studies

Experimental animals

Healthy male wistar albino rats of weight 150-200 g and female wistar albino rats of weight 140-200 g were used in this study. The animals were kept in well ventilated animal house conditions with free access to pelleted food and *ad libitum* water throughout the experiment.^[15]

Acute Oral Toxicity Study

The systemic acute oral toxicity (LD_{50}) profile of the isolated piperine was evaluated in female wistar albino rats according to OECD 425 guidelines. In brief, this method was carried out in three steps, the initial investigation in which nine animals were used, three animals per treatment group. The animals used were fasted overnight, note down the fasted body weights and calculate the doses, the dose volume should not be exceeded 1ml/100gm. The different doses selected were 500, 1000, 2000 mg/kg of the isolated piperine per body weight. Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter for a total of 14 days. However, the duration of the observation period should not be fixed rigidly. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary.^[16]

Evaluation of Anti-Inflammatory Activity

Carrageenan induced Hind paw edema in Albino Wistar rats

Albino Wistar rats of either sex weighing 150– 200 g were maintained in animal house and they were divided in to 4 groups of 6 animals each. Prior to the experimentation they were acclimatized to housing conditions for at least one week period of time to adjust to the new environment providing with food and water and *ad libitum*. In order to avoid the influence of diurnal variation, all the experiments were carried out at same time of the day i.e. between 9 a.m. to 5 p.m. Institutional animal Ethical Committee approval was obtained before carrying out this experiment.

Grouping and treatment

Group I--Control, animals were treated with 10% Tween-80 *p.o*

Group II--Standard group, animals were treated with 10mg/kg Indomethacin

Group III--Animals were treated with 30mg/kg b wt. *p.o* of isolated piperine from *Sindapsus officinalis* fruits.

Group IV-- Animals were treated with 60mg/kg b wt. *p.o* of isolated piperine from *Sindapsus officinalis* fruit.

Procedure

After 60 minutes of the respective treatments, Carrageenan (0.1ml of 1% w/v) was injected into sub plantar region of right hind paw. Paw volume was measured every hourly interval for a maximum of six hours by using mercury plethysmograph. Reduction in the paw volume was compared with the vehicle control.^[17]

RESULTS AND DISCUSSION

Table 1: Photochemical investigation.

S.No.	Test	Hydro alcoholic extract
1	Carbohydrates	
	Benedicts test	+
	Fehling's test	+
2	Proteins	
	Biuret test	+
	Millons test	–
3	Amino acids	
	Ninhydrin test	–
	Tyrosine test	–
4	Alkaloids	
	Mayers test	+
	Dragendroffs test	+
5	Glycosides	
	Borntragers test	–
6	Flavonoids	
	Lead acetate test	+
7	Phytosterols	
	Salkowski test	+
8	Fats and oils	
	Solubility test	–
	Stain test	–
9	Phenolics and tannins	
	Lead acetate test	+
	Acetic acid test	+
10	Volatile oils	
	Solubility test	+

(+) indicates present and (-) indicates absent

Table 2: Percentage yield of isolated piperine from *scindapsus officinalis* fruit.

S.no	Nature	Colour	%Yield
1.	Crystalline	Yellow	2.32% w/w

Table 3: Physicochemical standards of *scindapsus officinalis* schott fruit.

S.No	Parameters	Results
1	Loss on drying	2% w/w
2	Melting point	140 – 300 ⁰ C
3	P _H	7

Table 4: TLC of isolated piperine from *Scindapsus officinalis* fruits.

S.no	Composition of chemicals	Spot colour	Ratio	R _f value
1	Chloroform : methanol	Yellow	8:2	0.84
2	Ethyl acetate : methanol	Yellow	9:1	0.80
3	Benzene : ethyl acetate	Yellow	8:4	0.42
4	Acetic acid : methanol	Yellow	8:2	0.60

TLC of isolated piperine from *Scindapsus officinalis* fruits

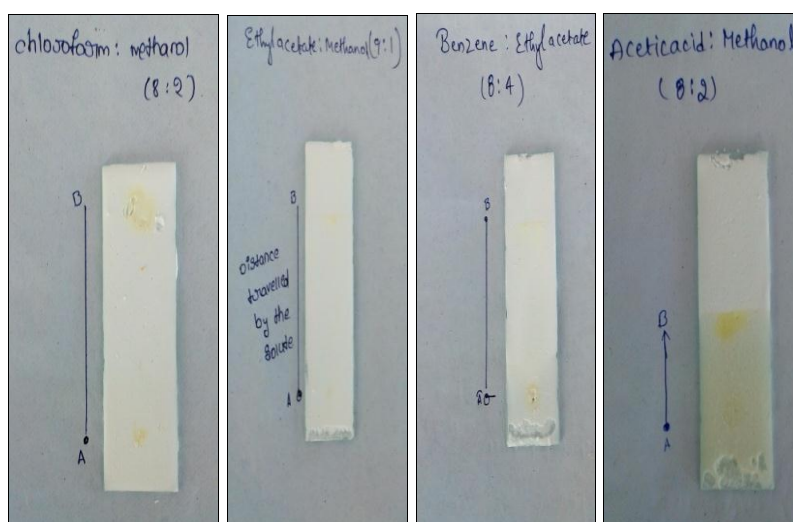


Fig-4

Fig-5

Fig-6

Fig-7

Chloroform: Methanol Ethylacetate: Methanol Benzene: Ethyl acetate Acetic acid: Methanol (8:2) (9:1) (8:4) (8:2)

Acute Oral Toxicity Study^[18]

For the LD₅₀ dose determination, isolated piperine from *scindapsus officinalis* was administered up to dose 2000 mg/kg body weight and extract did not produce any mortality, thus 1/5th, 1/10th, 1/20th of maximum dose tested were selected for the present study.

LD₅₀ of isolated piperine from *scindapsus officinalis* fruit was found to be –2000 mg/kg.

Table 5: Evaluation of Anti-Inflammatory Activity of *Scindapsus Officinalis* Fruits In Rats At 4 Hours.

S.No	Treatment	Paw Edema Volume(mm) Mean \pm SEM
1	Control (ascorbic acid)	4 \pm 0.23
2	Indomethcine (10mg/kg)	1 \pm 0.33*
3	High dose (400mg/kg)	1 \pm 0.14*
4	Low dose (100mg/kg)	2 \pm 0.24*

Values are Mean \pm SEM (n=6), Where, * represents significant at $p < 0.05$, when compared to Control group.

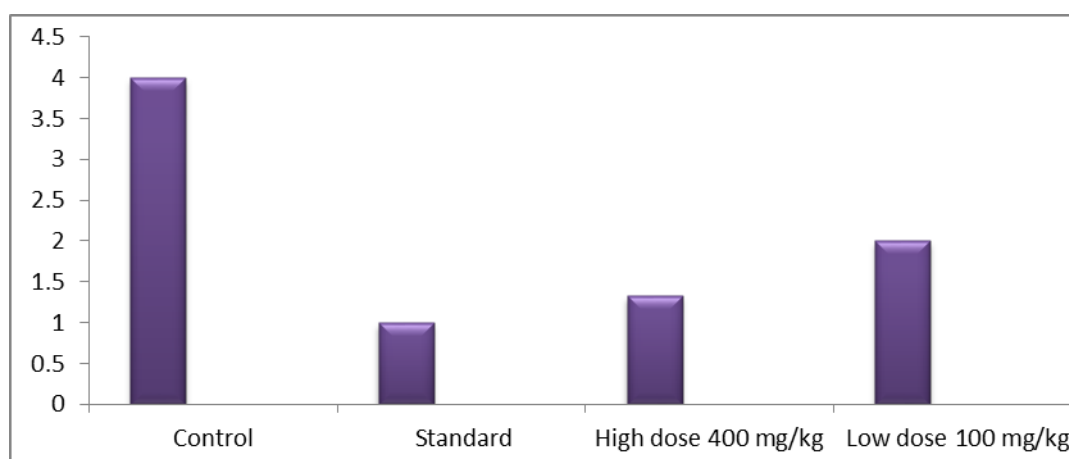


Fig-8: Evaluation of anti-inflammatory activity of *scindapsus officinalis* in rats at 4 hours.

Values are Mean \pm SEM (n=6), Where, * represents significant at $p < 0.05$, when compared to Control group.

DISCUSSION

There are a number of synthetic non steroidal anti inflammatory drugs (NSAIDs) currently available for use in the management, control and treatment of inflammation. However, most of the synthetic drugs are not only inaccessible and unaffordable, but also posses many toxic adverse effects therefore, there is a great need for the development of cheap, effective and safe NSAIDs from plants and other sources. In folklore medicine piperine is used in the treatment of inflammation. Based on its folklore application, the anti inflammatory activity of isolation of piperine was studied in carrageenan induced paw edema in rats. The inhibition of Carrageenan- induced inflammation in rats is an established model to screen compounds for

potential anti-inflammatory activity. It is well known that Carrageenan induced paw edema is characterized by biphasic event with involvement of different inflammatory mediators. In the first phase (during the first 2 h after Carrageenan injection), chemical mediators such as histamine and serotonin play role, while in second phase (3 – 4 h after Carrageenan injection). Kinin and prostaglandins are involved. Our results revealed that administration of isolation of piperine of fruit *scindapsus officinalis*. Inhibited the paw volume after third hour and during all phases of inflammation, which is probably inhibition of different aspects and chemical mediators of inflammation.

The Carrageenan induced leukocytes migration assay has been adjudged as an excellent acute and sub acute model for the measurement of fluid extravasations, leukocytes migration and other biochemical parameters which accompany inflammatory stimuli. Production of exudates in this model is related to local release of histamine, kinins and synthesis of prostaglandins. Migration of leukocytes would not be directly related to cyclo-oxygenase products, but the process is inhibited by non steroidal anti-inflammatory compounds indicating that many mechanisms may be implicated in its control. The isolation of piperine from *scindapsus officinalis* fruit prevented the formation of exudates and leukocytes mobilization induced by intraperitoneal injection of Carrageenan. The inhibitory effect of isolation of piperine from *scindapsus officinalis* fruit on the intraperitoneal formation of exudates and leukocytes mobilizations is probably due to the inhibition of prostaglandins. The gut wall contains prostaglandins E and F with prostaglandin synthetase activity mainly in the mucosa. Prostaglandin causes the intestinal cramps and diarrhoea which is due to effect on intestinal smooth muscle and secretion. Ricinoleic acid, the active principles present in the castor oil caused changes in mucosal cell layer permeability, electrolyte transport and intestinal peristalsis, leading to hyper-secretory response and diarrhea. It causes irritation and inflammation of the intestinal mucosa, leading to prostaglandin releases, which results in an increase in the net secretion of water and electrolytes in to small intestine.

Isolation of piperine from *scindapsus officinalis* fruit were subjected to 2 groups of (each group consists 3 animals). Carrageenan induced paw edema in rats at the intervals of 0 min, 30 min, 1 hr, 2 hrs and 4 hrs. Isolated piperine produces different rang of paw volume at different intervals. Incase of control animals, 3 animals' produces 5mm, 4mm and 4mm paw volume at 0min. At 30min it produces 5mm, 4mm and 4mm of paw volume. At 1hr it produces 5mm, 5mm and 3mm of paw volume. At 2 hrs it produces 5mm, 5mm and 4mm

paw volume and at 4hrs it produces 5mm, 4mm and 3mm of paw volume. In case of standard animals, 3 animals' produces 3mm, 4mm and 3mm paw volume at 0min. At 30min it produces 4mm, 3mm and 4mm of paw volume. At 1hr 3mm, 3mm and 3mm of paw volume. At 2 hrs it produces 3mm, 2mm and 2mm of paw volume and at 4hrs it produces 1mm, 1mm and 1mm of paw volume. In case of High dose animals, 3 animals' produces 3mm, 4mm and 3mm paw volume at 0min, at 30min 4mm, 4mm and 4mm of paw volume. At 1hr 3mm, 3mm and 3mm of paw volume. At 2 hrs it produces 2mm, 2mm and 2mm of paw volume and at 4hrs it produces 1mm, 1mm and 2mm of paw volume. In case of Low dose animals, 3 animals' produces 4mm, 4mm and 4mm paw volume at 0min, at 30min 3mm, 4mm and 3mm of paw volume. At 1hr it produces 3mm, 4mm and 3mm of paw volume. At 2 hrs it produces 2mm, 2mm and 2mm and at 4hrs it produces 2mm, 2mm and 2mm of paw volume.

After 4 hours treatment of standard, high and low dose of isolated piperine produces significant therapeutic response. So these data subjected to ANOVAs followed by Dennett's test. Animals treated with standard drug like piperine (10mg/kg) produces highly significant paw edema volume when compared to control animals. Whereas, high dose of 400 mg/kg isolation of piperine from *scindapsus officinalis* fruit produces highly significant decreased paw edema volume which is similar to the response of Piperine treatment. When compared to control animals. In case of low dose 200 mg/kg isolation of piperine from *scindapsus officinalis* fruit produces significant decreased paw edema volume when compare to control animals.^[28]

Isolation of piperine from *scindapsus officinalis* fruit showed potent anti-inflammatory activity may be due to the presence of flavonoids, phytosterols and tannins and also due to inhibition main inflammatory mediators like Histamine, serotonin, Prostaglandins, Bradykinin, Angiotensin, Trachykinin, platelet activating factor and substance-p Hence it is concluded at the Low dose possesses significant anti-inflammatory activity against carrageenan induced paw edema in rats.

CONCLUSION

The present study was carried out to find out the evaluation of anti-inflammatory activity of *scindapsus officinalis* fruit in rats. From the results we concluded that the *scindapsus officinalis* fruit isolate at high and low doses produces highly significant and significant decreased in carrageenan induced paw edema in rats. This activity may be due to presence of alkaloids, flavonoids, phytosterols and tannins in isolation of piperine from *scindapsus*

officinalis. However, long term studies in different animals and inflammation subjects may further substantial our study result.

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