Research Article

Anti-Inflammatory Activity of Methanol Extract of the Whole Plant of *Psychotria octosulcata*. W. A. Talbot

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ABSTRACT

In the present study, the anti-inflammatory effect of the methanol extract of the whole plant of *Psychotria octosulcata* was investigated. The methanolic extract of *Psychotria octosulcata* was ingested orally (p.o.) in the form of suspension in 0.5% Tween 80 in three different doses, (100,200 and 400 mg/kg body weight). The acute anti-inflammatory effect of *Psychotria octosulcata* was tested in carrageenan-induced paw oedema and chronic anti-inflammatory effect of *Psychotria octosulcata* was tested in cotton pellet granuloma pouch method in wistar albino rats and compared with the standard, diclofenac (40 mg/kg body weight). The results showed that *Psychotria octosulcata* has significant reduction ($p \leq 0.01$) in inflammation i.e. 67.94% (200 mg/kg body weight) in paw edema method and 72.61% (200 mg/kg body weight) in cotton pellet method as compared to the standard drug, diclofenac which was 69.23% and 74.85 respectively. These results indicate that the extracts could possess anti-inflammatory property. Both of the methods and the changes in the activities could be suggested as contributory effects to the use of *Psychotria octosulcata* in the management of inflammatory conditions.

Keywords: Anti-inflammatory, diclofenac, *psychotria octosulcata*, wistar albino rats

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INTRODUCTION

Inflammation is a complex reaction to injurious agents, such as microbes and damaged usually necrotic, cells that consist of vascular responses, migration and activation of leucocytes, and systemic reaction. Inflammatory reaction may cause hypersensitivity reaction to insect bites, drugs and toxin. Inflammatory reaction underlies some common chronic diseases such as rheumatoid arthritis, atherosclerosis and lung fibrosis (1). Inflammation is classified as Acute and Chronic. Acute inflammation is the initial response of the body to harmful stimuli, achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Chronic inflammation is prolonged inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process (2). Chronic inflammation will continue to stimulate pro inflammatory immune cells when they may not be needed. Systemic or chronic inflammation can have a domino effect arising from an immune system response that's out of control. Infection or injury triggers a chain of events known as an "inflammatory cascade". The familiar signs of normal inflammation, heat, pain, redness, and swelling, are the first signs that the immune system is being called into action. Inflammation begins when pro-inflammatory hormones in the body send

signals for white blood cells to come and clear out infection and damaged tissue. These agents are matched by equally powerful, closely related anti-inflammatory compounds, which move in once the threat is neutralized to begin the healing process. Pro-Inflammatory Hormones such as Prostaglandins are found within most tissues and organs that stimulate nerve cells, signaling pain to the brain. They swell the blood vessels at the injured site, opening space in the capillary walls for the white blood cells to enter. The blood and plasma rushing out of those enlarged vessels causes the swelling, tenderness, and redness. Prostaglandins also cause constriction as well as dilation of smooth muscle cells. Cytokines are immune system modulators produced by cells throughout the body. A subclass of cytokines known as leukotrienes (or interleukins) ensures that the immune response is checked before it begins to attack outlying healthy cells and tissue. Importantly, they call off the inflammatory response. Histamines are chemicals responsible for the itchy nose, watery eyes, or rash that often accompany an allergic reaction. Their function is to help remove whatever toxin is causing the problem (by sneezing, coughing, crying, and scratching). Histamines bring more blood and lymphatic fluid to the site of the invasion, which in turn carries white blood cells to the site and toxins away from it.

Figure 1: ***Psychotria octosulcata*** is a shrub belonging to the family *Rubiaceae* showed in **Figure 1** is the major group Angio-sperms (Flowering plants) grown in Southeast Asia. Biological activities of this plant that have not yet been studied nor reported. Literature review showed that the extracts of many *Psychotria* species showed anti-inflammatory and analgesic activity (3, 4&5), and preliminary tests pointed to alkaloids as major responsible for the effect. Preliminary phytochemical test of this plant showed presence of alkaloids. Based on the chemical test and literature review our aim was to evaluate the anti-inflammatory activity of methanolic extract of the whole plant of *Psychotria octosulcata* in rats.

MATERIALS AND METHODS

Drugs and Chemicals

All drugs and chemicals were obtained from local sources and were of analytical grade.

Plant Materials

The whole plant of *Psychotria Octosulcata* was collected from Kolli Hills, Namakkal district, Tamil Nadu, India. The plant was authenticated by Dr.S.John Britto, The Rapinat Herbarium, St.Joseph's College, Trichy and voucher specimen was deposited in the herbarium of the department of Botany (Specimen no; RM 001).The whole plant material was dried in shade and powdered, 1kg was extracted with methanol in a Soxhlet extractor for 36 hr. Extract was evaporated under low pressure by using Buchi type evaporator

Animals

Adult male wistar rats weighing 200-250g were obtained from Thanthai Roever College of Pharmacy, Perambalur. They were maintained at standard housing conditions and fed with commercial diet and provided with water add libitum during the experiment. The institutional animal ethical committee permitted the study.

Acute Toxicity Study

Acute toxicity study was carried out using female Albino mice (25 -30 g) by Acute toxic class method as per OECD guidelines. The methanol extract of *Psychotria Octosulcata* (MEPO) was orally administered to different groups of rats at the doses of 5,50,300,2000 mg kg-1 body weight respectively. Animals were observed for 48 h to study the general behavior of animals, sign of discomfort and nervous manifestation. The MEPO was found devoid of mortality of animals at the dose of 2000 mg kg-1 body weight.

Experimental Design

Anti inflammatory activity was evaluated using acute and chronic inflammatory model. Acute anti-inflammatory activity was evaluated by carrageenan – induced rat paw oedema method and the chronic inflammatory activity was evaluated by cotton pellet granuloma model (6, 7&8)

Carrageenan Induced Paw Edema Method in Rats

Acute anti-inflammatory activity was evaluated by carrageenan – induced rat paw oedema as described by Turner et al. Wistar albino rats were divided into 5 groups (n=6). Acute inflammation was produced by injecting 0.1 ml of 1% carrageenan into sub-plantar surface of rat hind paw. The control group I received tween 80 (0.5%) 0.1mL.The test group II, III and IV received 100,200 and 400 mg/kg methanol extracts respectively by oral route. The standard group V received the drug diclofenac 40 mg/kg by oral route. All the suspensions were administered 30 minutes before carrageenan injection (0.1mL of 1%). The paw volume, up to the tibiotarsal articulation, was measured using a plethysmometer at 1, 2, 4, & 6 hrs.

Cotton Pellet Granuloma Method in Rats

The chronic inflammatory model was evaluated by cotton pellet granuloma method. Wistar albino rats were divided into five groups (n=6). The animals were anaesthetized with ether; the back skin was shaved and disinfected with 70% ethanol. An incision was made in the lumbar region. Subcutaneous funnels were formed by using blunted forceps and subsequently sterilized cotton pellets weighing 20 ± 1mg were implanted on either sides of the scapular region of each rat. Group I served as control and received the vehicle. The methanol extract 100,200 and 400 mg/kg was administered orally to group II, III and IV animals for 7 days. Group V animals received diclofenac at a dose of 40 mg/kg p.o for 7 days. On the eighth day, the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60°C, weighed and compared with control (Increment in the dry weight of the pellets is taken as a measure for granuloma formation). The percentage increase in paw edema of the treated groups was compared with that of the control and the inhibitory effect of the extract was studied. The relative potency of the methanol extract (Test) was calculated based upon the percentage inhibition of the inflammation.

$$\% \text{ inhibition} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Statistical Analysis

The results were expressed as Mean ± SEM of six animals from each group. The statistical analysis was carried out by one way analysis of variance (ANOVA) P value < 0.01 were considered significant.

RESULTS

Phytochemical screening

Phytochemical screening of methanol extract of *Psychotria Octosulcata* has been presented in Table 1. Preliminary phytochemical screening of extract has revealed the presence of alkaloids, glycosides, flavonoids, phenols and sterols.

Carrageenan-induced rat paw edema test in rats

The anti-inflammatory activity of was measured at the dose of 100,200 and 400 mg/kg b.w. Against acute paw oedema induced by carrageenan is summarized in table 2. The sub plantar injection of

carrageenan caused a time-dependent paw oedema in the rat. In carrageenan-induced

Table 1: Phytochemical Screening of the Methanol Extract of *Psychotria Octosulcata*

Plant constituents	Result
Alkaloids	+
Fixed oils	-
Sterols	+
Flavonoids	+
Tannins	-
Steroids	-
Glycosides	+
Mucilages	-
Gums	-
Phenols	+

paw oedema in rats, oral administration of MEPO (100,200 and 400 mgkg-1 p.o.) inhibited paw oedema dose-dependently at 1, 2, 4, and 6 hr after carrageenan injection which was comparable with the diclofenac sodium treated group. MEPO exhibited 58.97, 67.94 and 58.97 % of inhibition at the dose of 100,200 and 400 mg/kg b.w. respectively in carrageenan-induced rat paw oedema. In 100 mg /kg and 400 mg /kg the effect is only marginal. The dose 200 mg /kg of MEPO (67.94%) effectively inhibited paw oedema very close to diclofenac sodium (69.23%). As shown in Table. 2, MEPO showed significant inhibition of rat paw edema at 6 hr.

Table 2: Anti-Inflammatory Activity of Methanol Extract of *Psychotria Octosulcata* on Carrageen Induced Paw Oedema Method

Group (n=6)	Dose Mg/kg	Paw Volume (Mean ± Sem)	Paw edema volume in ml (Mean ± Sem) 1 Hour	2 Hour	4 Hour	6 Hour	% Inhibition at 6 hour
I. Control	0.5% Tween80	0.25 ± 0.03	0.44±0.02	0.58±0.06	0.66±0.05	0.78±0.03	----
II. MEPO	100 mg/kg	0.26 ±0.01	0.41±0.05	0.39±0.02	0.37±0.03	0.32±0.01	58.97
III. MEPO	200 mg/kg	0.25 ±0.02	0.37±0.03	0.33±0.01	0.30±0.03	0.25±0.02	**67.94**
IV. MEPO	400 mg/kg	0.26 ±0.02	0.39±0.03	0.37±0.01	0.35±0.03	0.32±0.02	58.97
V.Std Diclofenac Sodium	40mg/kg	0.24±0.02	0.36±0.03	0.32±0.05	0.29 ±0.02	0.24±0.01	**69.23**

Values are expressed as Mean ±SEM for 6 animals in each group. Group V compared with Group I (P<0.001). Group II, III and IV compared with Group V (P<0.01)

Cotton pellet granuloma method in rats

The extract has been found to reduce the weight of cotton pellet granuloma in a dose dependent manner (Table 3) in the cotton pellet induced model of inflammation in rats. The reduction in the weight of cotton pellet granuloma with different doses of extract 100, 200 and 400 mg/kg was found 46.56%, 72.61% and 58.82% respectively, However, the decrease in inflammation by *Psychotria Octosulcata* 200 mg/kg was comparable to diclofenac sodium, which reduced the weight of cotton pellet granuloma by 72.61 % .

Table 3: Anti-Inflammatory Activity of Methanol Extract of *Psychotria Octosulcata* on Cotton Pellet Granuloma Method

Group	Dose	Dry weight of Cotton Pellet in mg	% Inhibition
I Control	-	92.84 ±0.53	-
II MEPO	100mg/kg	49.61 ±0.56	46.56
III MEPO	200mg/kg	25.42 ±0.58	72.61
IV MEPO	400mg/kg	38.23±0.54	58.82
IV Std (Diclofenac Sodium)	40mg/kg	23.34 ±0.56	74.85

Values are expressed as Mean ±SEM for 6 animals in each group. Group V compared with Group I (P<0.001). Group II, III and IV compared with Group V (P<0.01).

DISCUSSION

The carrageenan induced paw oedema used to assess acute inflammation develops by release of inflammatory mediators such as histamine, kinin, bradykinin, and prostaglandins. Test groups showed a significant reduction of the carrageenan induced paw edema volume compared with the control group. The MEPO extract (200mg/kg) showed significant inhibition of rat paw edema at 6 hr. The cotton pellet-induced granuloma is widely used to assess chronic inflammation. In the present study, administration of *Psychotria Octosulcata* extract has been observed to inhibit the weight of wet cotton pellet in a dose dependent manner and the dose of 200 mg/kg exhibited inhibition of inflammation very close to the inhibitory effect of diclofenac sodium. It is well known fact that diclofenac sodium act by inhibiting the prostaglandins synthesis at the late phases of inflammation. The findings of the present study have confirmed that *Psychotria octosulcata* has potent anti-inflammatory activity and justify its use in traditional medicine to treat inflammatory diseases and further investigation is needed for isolation of compound responsible for anti inflammatory activity.

CONCLUSION

By the above experiment we conclude that the methanolic extract of the whole plant of *Psychotria octosulcata* shows good anti-inflammatory activity at 200 mg/kg in both models and it may be used for further investigations.

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