

**Anti-inflammatory and Antidiarrhoeal Activities of
Ethanol Extract of *Ipomea obscura***

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Abstract

Ipomea obscura (family: Convolvulaceae) is used for the treatment of inflammation and diarrhoeal disorders by tribals of Kunjapanai, Nilgiris district, Tamil Nadu (India). However, the anti-inflammatory and antidiarrhoeal activities of the plant have not been scientifically evaluated so far. The main aims of this study were to evaluate scientifically the anti-inflammatory and antidiarrhoeal activities of ethanol extract of *Ipomea obscura*. The anti-inflammatory and antidiarrhoeal activities

were evaluated by; carrageenan induced paw edema, cotton pellet granuloma in rats, castor oil induced diarrhoea and charcoal meal test in mice. The ethanol extract at singly dose of 2000 mg/kg, p.o. in mice did not show any behavioral changes or mortality. Thus the LD₅₀ of this plant extract was found to be more than 2000 mg/kg. The ethanol extract (250 and 500 mg/kg p.o.) were administered to the rats resulted, significant (p<0.05) inhibition of carrageenan induced rat paw edema and cotton pellet granuloma. The extract (250 and 500 mg/kg) dose dependently reduced the intestinal propulsion of charcoal meal and produced significant (p<0.05) protection against castor oil induced diarrhoea. Preliminary phytochemical investigation showed the presence of glycosides, saponins, flavonoids, tannins and steroids. These observations prove scientifically the anti-inflammatory and anti diarrhoeal properties of *Ipomea obscura*, and thus provides pharmacological support for the tribal use.

Key words: *Ipomea obscura*, anti-inflammatory activity, antidiarrhoeal activity,

Introduction

Ipomea obscura Linn. (Convolvulaceae) is found through out India and much seen in Nilgiris, Tamil Nadu. The native tribals of Nilgiris used this plant to treat inflammation and diarrhoea (1). Reported that decoction of *I. obscura* is used to treat diarrhoea by traditional healers in Uganda. There have been reports that a majority of the population particularly those living in villages depends on herbal medicines (2). The main aim of the study was to assess the anti-inflammatory, antidiarrhoeal and antimicrobial potentials of extracts made of leaves of *I. obscura*.

Material and methods

Plant material

Fresh *I. obscura* plant was collected from a various places of Nilgiris, Tamil Nadu state India between august and October. The plant was authenticated by B. V. Krishna, Department of Botany, Government First Grade College, Chickballapur, Karnataka (India). The specimen was deposited in herbarium with voucher number KVCPP: 101 in Department of Pharmacognosy, Sri K.V. College of Pharmacy, Chickballapur, Karnataka (India).

Extraction

The collected leaf material was cleaned; air-dried and made coarse powder. About is 100 g of the coarse powder was extracted with 500 ml ethanol by soxhlet apparatus. After extraction the extract was concentrated at reduced temperature and pressure using rotary evaporator and the yield was noted.

Experimental animals

Albino Wister rats weighing about 150-200 g and Swiss albino mice 15-25g of either sex were for the investigation. Animals were obtained from the Animal House, Department of Pharmacology, J.S.S. College of Pharmacy, Ootacamund, Tamil Nadu (India), were used. The animals were housed under standardized animal house conditions (temperature 25 ± 2 °C, photoperiod: 12 h light and 12 h dark

cycle and relative humidity: 50-55%). All animals had free access to pelleted food (Kamadenu Enterprises, Bangalore, India) and water *ad libitum*.

Toxicity studies

A limit test at one dose level of 2000 mg/kg body weight of ethanol extract of *I. obscura* was administered orally to three mice and the animals were observed for 6 h continuously for behavioral changes and thereafter daily for 14 days for mortality (3).

Anti-inflammatory and antidiarrhoeal studies

Carrageenan-induced paw edema

The method described by Winter *et al.*, (4) was used. Carrageenan (0.1 ml of 1 % suspension) was injected into the supplantar region of the right hind paw of each rat. Ethanol extract of *I. obscura* (250 and 500 mg/kg. p.o.) was administered to rats 1 h before carrageenan administration. Control rats received 1ml/kg tween 80, and indomethacine (5 mg/kg. p.o.) was used as reference drug. The volume of the injected paw of these rats was measured 1 h prior to the injection of carrageenan and 1 and 4 h after the injection using the Plethysmometer (UGO Basile S.R.L. Samitek Instrument, Italy). Mean increase in paw volume was noted and percentage of inhibition was calculated.

Cotton Pellet granuloma

The method described by Meier *et al.*, (5) and Niemegeers *et al.*, (6) with slight modification was used. Albinos rats were selected and randomly divided into four groups each contain 6 animals. A sterilized cotton pellet weighing 20 mg was introduced subcutaneously into rats under ethereal anesthesia. They were then treated orally with the ethanol extract of *I. obscura* (250 and 500 mg/kg body weight) for six consecutive days. Control animals received 1 ml/kg tween 80, and indomethacine (5 mg/kg p.o.) was administered to animals in the reference group. On the seventh day, the animals were subjected to a light ethereal anesthesia,

and the pellets were removed, dried overnight at 60°C, and weighed.

Castor oil induced diarrhoea

The method described by Awouters *et al.*, (7) was used. Mice were fasted for 24 h and housed in individual cages lined with white blotting paper. The ethanol extract of *I. obscura* (250 and 500 mg/kg. p.o.) was administered to mice. Control mice received 1 ml/100 g tween 80; loperamide (2 mg/kg p.o.) was used as reference drug. One hour later, castor oil (1 ml/100 g p.o.) was administered, and the animals were observed for 4 h for the number of wet feces produced.

Gastrointestinal Motility Test

The method described by Akah *et al.*, (8) with slight modification was used to test the effect of the extract on gastrointestinal motility. Swiss albino mice were divided into 4 groups, 6 animals each. The ethanol extract of *I. obscura* (250 and 500 mg/kg. p.o.) was administered to mice. Control rats received 10-ml/kg tween 80; loperamide (2 mg/kg p.o.) was used as reference drug. After 30 min, 1 mL of 10% charcoal suspension in 5% acacia solution was administered to each mouse orally. The animals were sacrificed after 20 min and the abdomen was opened. The small intestines were dissected out and placed on a clean surface. The distance traveled by the charcoal meal from the pylorus to ceacum was measured. The entire length of the small intestine was also measured for calculating percentage distance traveled by the charcoal plug along small intestine.

Statistical analysis

The Data were expressed as mean \pm S.E.M. The differences were compared using one-way ANOVA followed by Dunnett's test using PRISM software (version 4). The results were considered significant when $p < 0.05$.

Results

Toxicity studies

In this study, we observed no significant behavioral changes or mortality during the 14 days observation period. Thus oral dose LD₅₀ of *I. obscura* was greater than 2000 mg/kg in mice.

Carrageenan induced rat paw edema

The extract of *I. Obscura* (250 and 500 mg/kg) produced statistically significant ($p < 0.05$) dose dependent inhibition of the edema induced by carrageenan. The percentage inhibition of post carrageenan induced edema was 54.17 % and 66.6 % by 250 and 500 mg/kg of the ethanol extract, respectively (Table II).

Table 1. Effect of *Ipomea obscura* extract on Carrageenan induced paw edema in rats

Treatment	Dose (mg/kg, p.o.)	Mean increase in paw volume	Percentage of inhibition
Vehicle	Tween 80	1.2 ± 0.22	-----
Indomethacine	5	0.38 ± 0.12**	68.33
Ethanol extract of <i>I. obscura</i>	250	0.55 ± 0.1*	54.17
	500	0.40 ± 0.1**	66.6

Values are expressed as mean ± SEM (n=6), Values are statistically significant at * $p < 0.05$ and ** $p < 0.01$ compared with Tween 80 treatment (Dunnett's test)

Cotton pellet granuloma

The animals treated with ethanol extract *I. obscura* (250 and 500 mg/kg) showed significant ($p < 0.05$) reduction in the post implantation weight of the cotton pellets when compared with rats that received vehicle (Table III).

Table 2. Effect of *Ipomea obscura* extract on Cotton pellet granuloma in rats

Treatment	Dose (mg/kg, p.o.)	Weight of cotton pellets (mg)		Inhibition (%)
		Before implantation	After implantation	
Vehicle	Tween 80	20.0 ±1.4	40.2±2.7	-----
Indomethacine	50	20.3±1.2	23.2±1.9**	42.28**
Ethanol extract of <i>I. obscura</i>	250	20.5±1.1	38.5±3.1	4.22
	500	20.0±1.2	26.4±2.3**	34.32**

Values are expressed as mean ± SEM (n=6), Values are statistically significant at **p<0.01 compared with Tween 80 treatment (Dunnett's test)

Effect on castor oil induced diarrhea

The number of feces produced by mice given castor oil (0.2 ml/mouse) was significantly reduced with extract (250 and 500 mg/kg) pretreatment, when compared with mice that received tween 80 prior to castor oil administration (Table III).

Table 3. Effect of ethanol extract of *I. obscura* on castor oil-induced diarrhea in mice.

Treatment	Dose mg/kg orally	Number of mice with diarrhoea	Percentage of protection
Control	-----	8	0
Loperamide	2	0	100***
Ethanol extract of	250	3	62.5*
	500	1	87.5**

Values are expressed as mean ± SEM (n=8), Values are statistically significant at*** p<0.001, ** p<0.01 and * p<0.05 compared with control (Dunnett's test)

Effects on gastrointestinal motility

Gastrointestinal motility was assessed by calculating the percentage of distance traveled by charcoal meal through small intestine after administration of ethanol extract *I. obscura* (250 and 500 mg/kg). The results indicates that the both doses of *I. obscura* extract showed significant ($p < 0.01$) reduction in propulsion of charcoal meal through gastrointestinal tract as compared to control group (Table IV).

Table 4. Effect of ethanol extract of *Ipomea obscura* on Gastro Intestinal Motility by Charcoal Meal Method

Treatment	Dose (mg/kg, p.o.)	Mean intestinal length (cm)	% Distance traveled	Percentage protection
Control	Tween 80	45.3 ± 1.8	34.5 ± 1.1	76.15
Loperamide	2	42.3 ± 1.2	12 ± 1.2	28.36**
Ethanol extract of <i>I. Obscura</i>	250	45.52 ± 1.73	24.3 ± 1.37	53.40*
	500	48.2 ± 1.48	19.6 ± 2.29	38.59*

Values are expressed as mean ± SEM (n=6), Values are statistically significant at** $p < 0.01$ and * $p < 0.05$ compared with Tween 80 treatment (Dunnett's test)

Discussion

In this study, the ethanol extract of the leaves of *I. obscura* was found to exhibit anti-inflammatory activity by inhibiting the edema induced by the injection of carrageenan into the hind paw of rats. This test is used for predicting the efficacies of anti-inflammatory agents that act by inhibiting the mediators of acute inflammation (8). Carrageenan-induced paw edema is biphasic, the first phase is attributed to the release of histamine, 5-HT and kinins and the second phase is related to the release of prostaglandins (9). The ethanol extract is very effective in inhibiting carrageenan-induced edema.

Cotton pellet granuloma is chronic inflammation is a reaction arising when the acute response is insufficient to eliminate proinflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and the infiltration of neutrophils and exudation (10)(11), chronic inflammation occurs by means of the development of proliferative cells. These cells can be either spread or in granuloma form. The ethanol extract showed significant ($p < 0.05$) anti-inflammatory activity in cotton pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation (12).

I. obscura exhibited antidiarrhoeal activity was found to be comparable to loperamide, a drug widely employed against diarrhoeal disorders that effectively antagonizes diarrhea induced by prostaglandins and cholera toxin (13),(14). The pharmacological effect of loperamide is due to its antimotility and antisecretory properties (15). Castor oil causes diarrhoea through the active metabolite ricinolic acid (16). Ricinolic acid increases peristaltic activity. The mechanism of action of castor oil induced diarrhoea is through elevated prostaglandin biosynthesis (7), (17) and (18). Prostaglandins contribute to the pathophysiological function in the gastrointestinal tract (19). *I. Obscura* showed significant reduction in the castor oil induced diarrhoea indicating that the extract has the ability to inhibit the synthesis of prostaglandins. The preliminary phytochemical screening shows the presence of tannins and flavonoids in the extract. Flavonoids are known to modify the production of cyclooxygenase 1 and 2 (COX-1 and 2) and lipooxygenase (LOX) (20), (21). According to Robertson et al., (22) tannins bearing preparations are also used for arresting diarrhoea because of their ability to coagulate and precipitate proteinaceous material.

In conclusion, the studies shown that the inhibition of prostaglandins synthesis is major mechanism by which the plant extract exerts anti-inflammatory and potent antidiarrhoeal properties. Thereby providing scientific support

in using this plant for the treatment of inflammation and diarrhoeal disorders by the tribal people of Kunjapanai of Nilgiris, Tamilnadu (India).

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