Acta Pharmaceutica Sciencia
48: 11-17 (2006)

Pharmacological Screening of Root of *Operculina turpethum* and its Formulations

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Abstract

The root of Operculina turpethum is the chief ingredient in the Ayurvedic formulation viz. Avipattikara churna used for the treatment of gastric ulcer and related gastrointestinal disturbances. Its incorporation in the formulation is not scientifically studied. Hence, in the present study root and its formulations are investigated for anti-ulcer, anti-inflammatory and anti-diarrheal properties.

The root powder of Operculina turpethum and its formulations were studied for anti-ulcer activity by using Shay rat that have administered preparation at 30 mg/kg and 100 mg/kg dose levels. The results revealed that the root powder and formulations have reduced the hyperacidity to the extent of 50 – 55% at 100mg/kg dose level. The root powder was found to be better than all formulations. The root powder and the formulation have reduced the charcoal movement significantly in charcoal meal test in mice at 400mg/kg dose. Similarly, these preparations have reduced the edema volume in formalin induced inflammation model in rats at 100mg/kg dose.

The study revealed that the incorporation of root of Operculina turpethum in Avipattikara Churna is justifiable.

Key words: Avipattikara churna, diarrhea, edema volume, hyperacidity, Operculina turpethum, reduction and ulcer.

Introduction

Operculina turpethum is a perennial herb with milky juice and its root is incorporated in the Avipattikara churna (an Ayurvedic preparation used for the treatment of hyperacidity, gastric ulcer and related gastrointestinal disturbances). The formula of the Avipattikara churna is given in table 1. This churna is useful in the treatment of Amla Pitha (Hyperacidity), malabandha (Constipation) (Ayurvedic formulary of India, 1976). In ancient literature it is named Trivrit (Ogle, 1931) Operculina turpethum is the chief ingredient in the formulation. Zandu Pharmaceuticals, Bombay, India, Sandu Pharmaceuticals and many native practitioners are manufacturing and marketing the same product with generic names. Upon literature review, it was found that the active principle of the plant is glycosidic resin (Ambasta, 1986). The scopoletin, a coumarin derivative, turpethinic acid and its derivatives were isolated from the plant (Rastogi and Mehrotra, 1999). However, there are no reports regarding its pharmacological profile in the modern literature. Hence, in the present study it was planned to

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investigate the claims of the Ayurveda regarding the usage of *Operculina turpethum* and the Avipattikara Churna in treating hyperacidity and related gastrointestinal disturbances.

Materials and methods

Collection of Plant material

The root of the plant was obtained from crude drug market and was identified by the native practitioner who is prescribing the preparation for his patients and was further identified by Prof. Srivatsa, Head, Dept. of Botany, L.V.D. College, Raichur, India. Thus obtained plant material was powdered and stored in an airtight container. Similarly Marketed Avipattikara churna was purchased from the Ayurvedic medical hall and a formulation was obtained from the native practitioner himself.

Table–1: Formula of Avipattikara Churna

Each 10 g contains:

Sl. No.	Name of ingredients	Binomial name	Quantity in Gm
01.	Sunthi	*Zingeber officinalis*	0.075
02.	Kali mirchi	*Piper nigrum*	0.075
03.	Pippli	*Piper longum*	0.075
04.	Tiphala Churna		
	i. Amlaki	*Emblica officinalis*	0.075
	ii. Haritaki	*Terminalia chebula*	0.075
	iii. Bibhitaka	*Terminalia bellerica*	0.075
05.	Mustha	*Cyprus rotundeus*	0.075
06.	Varading	*Embelia ribes*	0.075
07.	Ellyachi	*Elettaria cardamomum*	0.075
08.	Lavang	*Syzygium aromaticum*	0.833
09.	Patra	*Cinnamomum tamala*	0.075
10.	Vida lavana	Sod. Sulfate	0.075
09.	Trivrit	*Operculina turpethum*	3.333
10.	Sugar	- - -	5.000

Animals: Acute toxicity and blind screening studies were carried out in healthy mice of either sex weighing between 25 – 35g. The anti-inflammatory and anti-secretory and anti-ulcer activities were studied in healthy rats of either sex weighing between 150-200g. Both species were obtained from the central animal house, V.L. College of Pharmacy, Raichur. The animals were randomly distributed into various groups and were kept in colony cages at ambient temparature of $27^0 \pm 2^0$ C and at 45 – 55% relative humidity with a 12 hour light /12 hour dark cycle.

Blind Screening and Acute toxicity studies

The screening method as described by Robert (1965) was adopted in the present study. Albino mice of either sex weighing between 25–35 g were fasted and divided into 29 groups of 6 animals each. All the animals were fasted for 24 hours prior to the experiment. Animals of the group 1 received acacia suspension (0.5 ml orally). The animals of group 2 to 8 received suspension of root of *Operculina turpethum* (OT) at 10, 30, 100, 200, 400, 600, 800 mg/kg dose levels respectively. Similarly animals of group 9 to 15 received AVIFN (Avipattikara Churna from native practitioner), animals of group 16 to 22 received AVIFS (Avipattikara Churna from Sandu Pharmaceuticals) and animals of group 23 to 29 received AVIFZ (Avipattikara Churna from Zandu Pharmaceuticals) respectively at the dose levels mentioned above. The animals were observed at 0, ½, 1, 2 and 4 hours after the administration for acute effect and mortality. The observation was continued for one week for the delayed effects and mortality. However there were no deaths in any of the groups.

Antisecretory and Ulcer protective activity

Albino rats of either sex weighing between 150 – 200 g were divided into 11 groups of 6 animals each. These animals were fasted for 24 hours and water ad libitum, prior to experiment. Animals of groups 1 and 2 received 5% acacia suspension and omeprazole 30 mg/kg respectively. Similarly animals of groups 3 and 4 received *Operculina turpethum* 30 mg/kg and 100mg/kg, respectively. The animals of groups 5 and 6 received AVIFN, 7 and 8 received AVIFZ, 9 and 10 received AVIFS equivalent to 30mg/kg and 100 mg/kg of *Operculina turpethum* orally, respectively. The animals of group 11 received 100 mg/kg of Avipattikara churna (AVIFN) without *Operculina turpethum* (AVIFN – OT). One hour after the administration of the drugs, ulcers were induced by pyloric ligation (Shay *et al.*, 1945). After six hours the animals were sacrificed by decapitation. Abdomen was opened, oesophageal end was tied and the stomach was isolated. The contents of the stomach were collected and gastric volume, free acid and total acid content were estimated by titrimetric method. The stomach was opened; ulcer index was determined (Kulkarni, 1987) by observing under 10X lens and by giving scores (normal colored stomach = 0, red coloration = 0.5, spot ulcers = 1, hemorrhagic streaks = 1.5, ulcer ≥ 3 but $\leq 5 = 2$ and ulcers $\geq 5 = 3$). The results are compiled in table 2.

Anti-diarrheal activity

Mice of either sex weighing between 20 – 30 g were divided into 4 groups of 6 animals each. These animals were fasted for 24 hours prior to experiment. Animals of group 1 received 0.5 ml of 5% acacia suspension, group 2 received loperamide HCl 5 mg/kg, and groups 3 and 4 received *Operculina turpethum* and AVIFN 400 mg/kg orally, respectively. One hour after the administration of drugs 0.3 ml of 5% charcoal suspension (charcoal meal) was given to all the animals (Robert, 1965). Fifty minutes after the charcoal meal, all animals were sacrificed by decapitation. Abdomen was opened and the total length of the intestine was measured. The distance traveled by the charcoal meal in the intestine was also measured. The % movement of the charcoal meal in the intestine with reference to the total length of the intestine was calculated. The results are compiled in table 3.

Anti-inflammatory activity

The albino rats of either sex weighing between 120 – 160 g were selected and divided into 6 groups of 6 animals each. The animals were fasted for 24 hours prior to the experiment. Animals of groups 1 and 2 were treated with 0.5 ml of 5% acacia suspension and diclofenac sod., 30mg/kg respectively. Similarly, animals of groups 3, 4, 5 and 6 were treated with

Operculina turpethum, AVIFN, AVIFS and AVIFZ 100mg/kg respectively. One hour after the drug treatment all animals of all groups were given 0.1 ml of formalin subcutaneously into the subplantar region of the left hind paw. Paw volumes were measured at 0, ½, 1, 2, 4, 6, 8, 12, 18 and 24 hours after the formalin challenge by using plethysmograph (Kulkarni, 1987). Edema volumes were calculated and % reduction in the edema volume due to drug treatment was determined by comparing with control group (i.e. group1). The results are compiled in table 4.

Results and Discussion

All the preparations studied have shown dose dependent stimulation in the animals however the plain OT was found to be more effective among all the preparations. None of the preparations were found to be toxic in mice up to the dose of 800 mg/kg. And hence, LD_{50} of the preparations could not be calculated. But it is presumed to be above 800mg/kg dose. 30mg/kg and 100mg/kg doses have shown significant activity in blind screening, therefore these doses were opted for further studies.

All preparations viz. OT, AVIFN, AVIFS and AVIFZ were found to reduce the acid content (both free acid and total acid), but failed to reduce the volume and ulcer index significantly with single bolus injection in Shay rat with the preparations at both dose levels tried i.e. 30 mg/kg and 100 mg/kg. OT was found to be more effective in this case also. Since, LD_{50} is more than 800 mg/kg and is 5 times more than that of effective dose (100 mg/kg), we can infer that the root powder and its marketed formulations are safe for treating hyper acidity and gastric ulcer.

Table–2: Antisecretory and Ulcer protective activity of *Operculina turpethum* and Avipattikara churna

Drug	Dose Mg/kg	Free acid mEq/l/100g	Total acid mEq/l/100g	Acid volume ml/100g	Ulcer index
5% Acacia suspension (Control)	0.5 ml	84.4 ± 1.2	90.8 ± 1.9	3.9 ± 0.1	4.6 ± 0.2
Omeprazole (Std.)	30	04.9 ± 0.4*	07.2 ± 0.3*	1.2 ± 0.1*	1.3 ± 0.2*
OT	30	55.2 ± 0.4*	59.9 ± 0.8*	3.1 ± 0.3	3.6 ± 0.2
	100	48.5 ± 0.5	52.2 ± 0.5	3.0 ± 0.4	3.5 ± 0.2
AVIFN	30	67.3 ± 1.3*	73.0 ± 1.2*	4.7 ± 0.3	3.8 ± 0.3
	100	53.1 ± 0.6*	57.6 ± 0.4*	4.8 ± 0.2	3.5 ± 0.2
AVIFS	30	62.8 ± 0.5*	66.4 ± 0.5*	4.1 ± 0.2	3.6 ± 0.3
	100	54.3 ± 0.3*	60.0 ± 0.3*	3.6 ± 0.2	3.5 ± 0.2
AVIFZ	30	72.7 ± 2.9	81.0 ± 3.0	6.9 ± 0.3	4.3 ± 0.2
	100	63.9 ± 2.7*	69.4 ± 2.1*	4.8 ± 0.3	3.6 ± 0.2
AVIFN – OT	100	81.8 ± 0.5	85.6 ± 0.7	6.8 ± 0.5	4.3 ± 0.2

* Significant at P = 0.05

Pilot studies on charcoal meal test in mice revealed that lower doses like 30 mg/kg, 100 mg/kg and 200 mg/kg could not influence the intestinal movement. Hence, 400mg/kg dose was selected. Single bolus injections of OT and AVIFN have reduced the intestinal movement from 75.1 ± 1.1 to 56.0 ± 1.5 and 67.5 ± 2.1 respectively. The results of charcoal meal test reveals that, the dose required was higher (400mg/kg) than the dose required for reducing the hyperacidity. In addition the exact LD_{50} of the OT/formulations are not established. Therefore, using these preparations for antidiarrheal purpose require further studies to confirm their safety.

Table–3: Antidiarrheal activity of *Operculina turpethum* & Avipattikara churna

Drug	Average Total length of git (in cm)	Average length moved by marker (in cm)	% movement of marker
Control 5% Charcoal suspension	51.92 ± 1.77	39.25 ± 1.44	75.1 ± 1.1
Loperamide HCl 5 mg/kg Std.	64.00 ± 2.28	28.50 ± 1.33	44.5 ± 1.6*
OT 400 mg/kg	59.08 ± 2.87	32.92 ± 0.88	56.0 ± 1.5*
AVIFN 400 mg/kg	64.00 ± 1.31	43.17 ± 1.19	67.5 ± 2.1*

* Significant at P=0.05

The pretreatment with the root powder of OT, and marketed Avipattikara churna viz. AVIFN, AVIFS and AVIFZ (100 mg/kg dose) have reduced the formalin induced edema volume to the extent of 36.45%, 27.11%, 18.69% and 21.15% respectively. All these preparations have demonstrated significant anti-inflammatory activity; therefore they are useful in hyperacidity and ulcer related bowel inflammation also

Table – 4: Antiinflammatory activity of *Operculina turpethum* and Avipattikara churna

Drug	Mean % reduction in edema volume								
	½ hr	1 hr	2 hr	4hr	6hr	8hr	12 hr	18 hr	24 hr
Control 0.5ml 3% acacia suspension	--	--	--	--	--	--	--	--	
Diclofenac Sod. Std. 30 mg/kg	21.0±9.6	29.2±7.5	44.5±6.5	60.4±3.8	36.3±5.2	29.0±6.3	25.7±4.9	21.3±4.3	23.3±5.0
OT 100 mg/kg	11.4±4.0	12.9±2.8	25.7±7.0	36.4±8.5	28.7±7.5	20.1±5.5	15.7±4.3	19.0±4.9	28.5±8.9
AVIFN 100 mg/kg	08.9±5.6	10.8±7.1	17.8±4.8	24.8±5.7	27.1±7.5	15.5±6.5	14.7±3.5	16.0±3.5	20.4±7.8
AVIFS 100 mg/kg	0.0	07.5±4.7	17.0±6.1	18.6±11.4	16.9±10.9	11.1±11.1	08.9±4.8	10.9±5.5	26.1±6.6
AVIFZ 100 mg/kg	05.8±5.5	13.0±8.7	19.0±9.3	21.1±4.6	20.1±7.1	14.6±6.8	14.3±25.4	19.7±4.7	35.1±8.5

Avipattikara churna is normally given for a chronic period in treating hyperacidity, ulcer and related bowel disturbances. But in the present study only single bolus dose was given and observed the effect of the preparations was observed on only one model of hyperacidity. The effects of chronic administration on this model and on different models of hyperacidity are being planned. It is also planned to explore the possible mechanism of action of these preparations in reducing the gastric acid content. However the present study has indicated that OT and its marketed preparations (Avipattikara churna) are effective in reducing hyperacidity and related git disturbances.

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Received: 04.01.2005
Accepted: 30.12.2005