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Original Article

Anti-inflammatory activity of methanolic extract of *Corchorus depressus (L.)* Stocks

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ABSTRACT

The present study describes screening for anti-inflammatory activity of alcoholic extracts of *Corchorus depressus (L.)* Stocks. The plant has been used in the traditional Indian system of medicine as an antibacterial, antifungal and anthelmintic drug, as a tonic, cooling medicine in fevers; its mucilage is prescribed in gonorrhoea. Root is rubbed on stone and smeared over forehead to get relief in migraine; extract of plant is applied as a paste in healing of wounds and has anti-inflammatory activity. Methanolic extract of whole plant was studied for its *in-vivo* anti-inflammatory potential using Carrageenan induced rat paw edema and cotton pellet induced granuloma methods. The results of the study indicate that the alcoholic extract possess significant anti-inflammatory activity at doses 150 mg/kg and 300 mg/kg and justifies the folklore claim of utilisation.

1. INTRODUCTION

Inflammation is defined as a local response of living mammalian tissue to injury due to any agent. It is a body defence reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necropsied cells and tissues [1]. A large number of Indian medicinal plants are attributed with various pharmacological activities because they contain a diversified class of phytochemicals. It is believed that current analgesia-inducing drugs such as opioids and non-steroidal anti-inflammatory drugs are not useful in all cases, because of their side effects and potency [2]. Several plants have been used in folklore medicine as anti-inflammatory agents and they play an important role in health services around the globe [3].

Corchorus depressus (L.) Stocks commonly known in Sanskrit as Bedani; Odiya as Bojoromuli, is a perennial herb. The plant is treated as sacred and worshipped by the married women of Odisha, India, in the rituals called as **"Jama Jutia"**; the traditional ancient method of worshiping the Lord **"Yamaraj"** (The Lord of Death) following which they softly beat their family members with the worshiped plant and it is believed that by doing so the family members will be free from attack of any disease and have a long life. (Fig. 1)

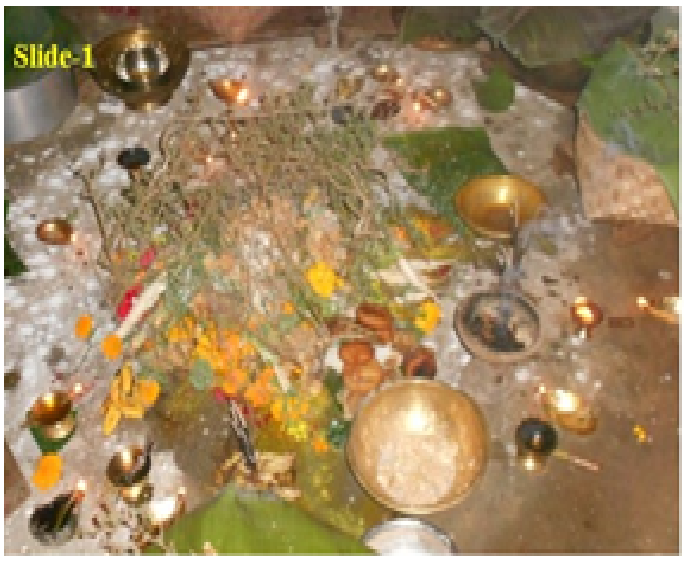

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Fig. 1. The photographs of the plant, worshipped by the people of Odisha in the festival 'JAMAJUTIA' (Slide 1, 2 &3)

The plant has been used in the Indigenous system of medicine as a tonic, cooling medicine in fevers; its mucilage is prescribed in gonorrhoea. Root is rubbed on stone and smeared over forehead to get relief in migraine [4]. It is also used to increase the viscosity of seminal fluid, to set-up menstrual disorder [5]. An extract of plant is applied as a paste in healing of wounds [6]. It has been used as antibacterial, antifungal, anthelmintic drug in Folklore medicine [7], as antimalarial [8], has cardio tonic activity [9], as tonic [10], in treatment of gonorrhoea [11], as veterinary medicine [12] and possesses diuretic activity [13]. Lack of scientific data with respect to the pharmacological properties of *Corchorus depressus* (*L.*) *Stocks* encouraged for the evaluation of its anti-inflammatory potential.

2. EXPERIMENTAL

2.1 Plant material

Corchorus depressus (L.) Stocks were collected from the coconut gardens of Salipur, Odisha in the month of August 2009. The plant was identified, authenticated by Botanical Survey of India, Central National Herbarium, Howrah (No- CNH/I-I/28/2009/Tech.II/93) and a voucher specimen was kept in the herbarium of Sri Jayadev College of Pharmaceutical Sciences, Naharkanta, Bhubaneswar, Odisha. (Slide 4-7)

Plant photos

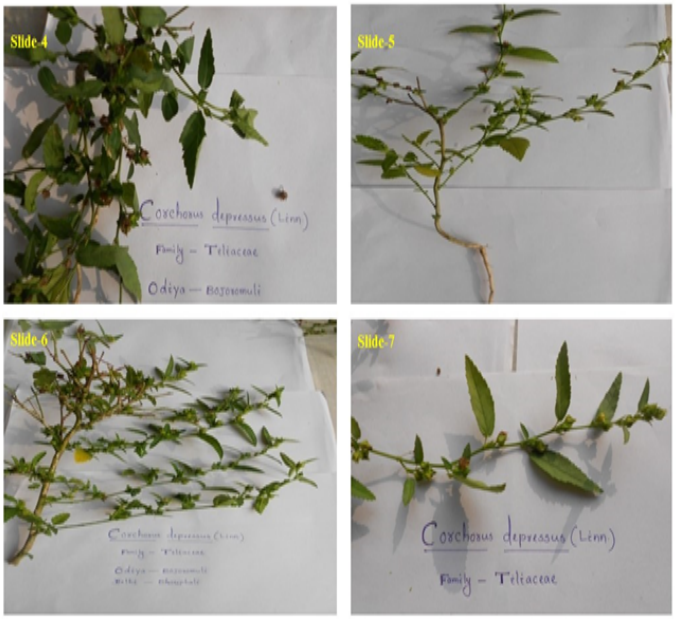

Fig. 2. Photographs of the whole Plant *Corchorus depressus (L.) Stocks* [Slide 4-7]

2.2 Preparation of extract

The whole plants were collected and washed thoroughly in water, chopped, air dried for a week at 35-40^0 C and pulverized in electric grinder. 150 gm of the powder subjected to Soxhlet apparatus using methanol as solvent. The solvent was then removed under reduced pressure, which obtained a greenish-black coloured residue. The yield was 5.4%. The prepared extract was used for the anti-inflammatory activity. The prepared extract was used for phytochemical screening [14] and evaluation of anti-inflammatory activity.

2.3 Experimental animals

Healthy adult Wistar rats, weighing about (150-200g) and Swiss albino mice of either sex (20-25g.), were procured from the Central Animal House, Sri Jayadev college of Pharmaceutical Sciences Naharkanta, Odisha and were used for the study. The animals housed in polypropylene cages with stainless steel lid and maintained under standard conditions (12 hr light/12 hr dark cycle; temperature 25±3˚C; 30-70% humidity) were fed certified rodent pellet diet from Nutrilab (Tetragon Chem. Pvt. Ltd., Bangalore) and purified tap water ad libitum. All the rats were acclimatized to the laboratory conditions for at least 4 days prior to the experiment. Before each test, the animals were fasted for at least for 12 hours. The guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA) Govt. of India were followed and prior approval was taken from the institutional animal ethics committee (IAEC) for conducting the animal experimental studies. (678/02/A/CPCSEA). All the

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experiments were performed in the morning according to current guidelines for investigations of experimental pain in conscious animals. [15]

2.4 Chemicals

Anaesthetic ether from Kabra Drugs Limited, Indore, Absolute alcohol, Hayman, England and sterillium disinfectant solution was procured from Bode Chemie Hamburg, Germany.

2.5 Experimental design

2.5.1 Carrageenan induced rat paw edema [16]

In each group six Wistar rats were taken. The animals were kept fasted throughout the experimental period, but were provided with water ad *libitum*. After 30 minutes 0.1 ml Carrageenan (1%) was injected into the planter region of hind paw of rats. Measurement of paw volume (ml) was made by mercury displacement technique using Plethysmometer immediately before and 3hr after carrageenan injection.

Group-I: Control [Animals received Carboxy methyl cellulose (100mg/kg P.O.)]

Group-II: Standard [Indomethacin treated Animals (10mg/kg I.P.)]

Group-III: Test-1 [Methanolic extract of *Corchorus depressus (L.) Stocks* (150mg/kg)]

Group-IV: Test-2 [Methanolic extract of *Corchorus depressus (L.) Stocks* (300mg/kg)]

2.5.2 Cotton pellet induced granuloma [17]

The animals were divided in to four groups (n=6). The animals were anaesthetized with ether; the back skin was shaved and disinfected with 70% ethanol. An incision was made in lumbar region. Using a blunted forceps, subcutaneous tunnels were formed and sterilized cotton pellets weighing 20±0.5 mg were implanted on either sides of the scapular region of each rat. Group-I served as control and received the vehicle.

The methanolic extract of *Corchorus depressus (L.)* Stocks at doses of 150 mg/kg and 300 mg/kg were administered orally to Group-II and Group-III animals respectively for 7 days. Group-IV animals received Indomethacin at a dose of 10 mg/kg p.o. for the same period. On the 8th day, the animals were sacrificed and the pellets together with the granuloma tissue were carefully removed, dried in an oven at 60^0 C, weighed and compared with the control. The percentage activity of anti-inflammatory effect of methanolic extract of *Corchorus depressus (L.)* Stocks was calculated by the following formula:

$$\text{Percentage inhibition} = (C - T)/C \times 100$$

Where, T is increase in paw volume after administration of test extract and C is increase in paw volume of control group.

2.5.3 Egg albumin induced paw edema

The experiment was conducted by inducing egg- albumin to healthy rats [18]. All four groups were pretreated with vehicle or Indomethacin or the alcoholic extract at both doses (150 mg/kg, 300 mg/kg) respectively. After 30 min, each group was injected with 0.5ml raw egg albumin sub-plantar to the left hind paw. The paw volume compared to that of the control animals was recorded at 0, 1, 3, 6, 12 hr and considered as anti-inflammatory response.

2.5.4 Statistical evaluation

The data were statistically analysed by student's t-test and all the values were expressed as mean± SEM. The data were also analysed by one way ANOVA followed by Dunnet's t-test and values P<0.05 were considered significant. [19]

3. RESULTS AND DISCUSSION

Administration of Carrageenan in paw edema of rats produced a short inflammatory response indicated by increase in paw volume. Oral administration of methanolic extract of *Corchorus depressus (L.)* Stocks showed a significant (P<0.05) inhibition of Carrageenan induced paw inflammation at dose 150mg/kg (34% inhibition) and at 300 mg/kg (42% inhibition). (Table 1)

Table 1: Effect of methanolic extract of *Corchorus depressus (L.)* Stocks on Carrageenan induced paw edema in Rats

Group No. (n=6)	Design of Treatment	Dose (mg/kg)	Increase in paw edema at the end of 3hr.	Percentage of Inhibition
I	Control	–	0.48±0.03	–
II	Indomethacin	10	0.22±0.01**	57
III	Test-1 *C.depressus*	150	0.32±0.05**	34
IV	Test-2 *C.depressus*	300	0.29±0.02**	42

**P<0.01 Vs control. The data were statistically analyzed by Student's t-test and all values were expressed as Mean± SEM. The data were also analyzed by one way ANOVA followed by Dunnet's t-test and values P< 0.05 were considered significant.

Table 2: Effect of extract of *C. depressus* in cotton pellet induced granuloma model

Group No. (n=6)	Design of Treatment	Dose (mg/kg)	Weight of cotton pellet (mg)	Percentage of Inhibition
I	Control	-	70.3±0.6	-
II	Test-1 *C.depressus*	150	48.8±0.5*	32
III	Test-2 *C.depressus*	300	30.51±0.8*	44
IV	Indomethacin	10	26.32±0.6*	55

*P<0.05 Vs control. The data were statistically analysed by Student's t-test and all values were expressed as Mean± SEM. The data were also analysed by one way ANOVA followed by Dunnet's t-test and values P< 0.05 were considered significant.

In cotton pellet induced granuloma the percentage inhibition (44%) at 300 mg/kg was found significant and comparable to Indomethacin at 10 mg/kg (Table 2). The effect of methanolic

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extract of *Corchorus depressus (L.) Stocks* was evaluated on egg-albumin induced rat paw edema. It was observed that the test compound at dose 300 mg/kg produced a significant reduction in the paw volume at 3, 6 and 12 hr, which is comparable with the standard drug Indomethacin (10 mg/kg). (Table 3)

Table 3: Effect of Petroleum ether extract of *C. depressus* on egg-albumin induced rat paw edema

Groups (n=6)	Dose (mg/kg)	Paw Volume (ml)				
		0 hr	1 hr	3 hr	6 hr	12 hr
Control	1% CMC	1.27±0.01	1.82±0.02	2.41±0.01	1.50±0.02	1.42±0.01
Indomethacin	10	1.26±0.01	1.73±0.01**	1.38±0.02***	1.18±0.00***	1.14±0.02***
Test-1 C.depressus	150	1.24±0.01	1.82±0.01	1.63±0.02***	1.47±0.01	1.39±0.00
Test-2 *C.depressus*	300	1.25±0.02	1.77±0.01	1.50±0.01***	1.25±0.01***	1.27±0.02***

**P<0.01, *** P<0.001 Vs control.

The data were statistically analyzed by Student's t-test and all values were expressed as Mean± SEM. The data were also analyzed by one way ANOVA followed by Dunnet's t-test and values P< 0.05 were considered significant.

The phytochemical studies on the *Corchorus depressus (L.)* Stocks extract revealed the presence of various constituents like alkaloids, Flavonoids, carbohydrates, glycosides, phenols and tannins (Table 4).

Table 4. The preliminary phytochemical studies for testing different chemical groups present in methanolic extract.

Plant constituents		Results	Inference
Tests/Reagents used			
1.	Glycosides		
	Borntrager's Test: To 3ml of extract dil H_2SO_4 was added and filtered + equal volume of chloroform added. Organic solvent separated and ammonia added	Ammonia-layer turned to pink	+
2.	Phenolic Glycosides		
	Ferric sulphate Test: 10 ml of methanolic extract is evaporated to dryness and the residue is dissolved in 10 ml of water. To 1ml of aqueous solution, a crystal of ferric sulphate is added	At first violet colour appears and then a precipitate is formed	Presence of phenolic glycosides
3.	Alkaloids		
	Dragendorff's Test: Alcoholic extract was evaporated and dil. HCl was added shaken and filtered. Few drops of dragendorff's reagent added to it	Orange brown ppt. found	+
4.	Flavonoids		
	(*i*) Shinoda Test: To dry powder of extract 5ml of 95% ethanol was added and with few drops of HCl & 0.5 g. of magnesium turning were added	Pink colour was observed	+
	(*ii*) Lead acetate : Lead acetate was added to small quantities of extract	Yellow colour observed	+
	(*iii*) Sodium hydroxide Test : To the residue sodium hydroxide was added	Yellow colour observed	+
5.	Carbohydrate		
	Molish's Test: To the 2-3 ml of aqueous extract few drops of α-napthol was added, shaken then con. H_2SO_4 was added from sides of the test tube	Violet ring formed at the junction of two liquid	+
6.	Tannins		
	(i) Ferric Chloride Test: 2 ml of alcoholic extract is diluted with 3 ml of water and 3 drops of dilute ferric chloride is added	Green black colour	Presence of catechol (condensed tannins)
	(ii) NaCl Test: 2 ml of alcoholic extract is diluted with 3 ml of water and treated with 5 ml of 2% NaCl solution. Precipitate is formed and filtered through filter paper and 5 ml of 1% gelatin added which gives precipitate.	Precipitate disappears after addition of excess gelatin	+

7.	Test For Phenol		
	Methanolic Ferric chloride Test: To the 3ml of alcoholic extract 5% methanolic ferric chloride was added	Deep blue colour formed	+

4. CONCLUSION

The methanolic extract of *Corchorus depressus (L.) Stocks* showed significant anti-inflammatory activity against carrageenan and egg-albumin induced paw edema and cotton pellet induced granuloma models in rats. The anti-inflammatory activity may be attributed to the presence of different phytoconstituents present in the plant extract, especially flavonoids, which are found to act by reducing the release of inflammatory substance like prostaglandin there by reducing tissue exaggeration [20]. Further detailed investigation needs to be underway to determine the exact phytoconstituents, which are responsible for the anti-inflammatory activity and may provide deeper insight to the discovery of a potent drug for the treatment of inflammation. The inhibitory activity of the extract justified the use of the plant as a non-specific anti-inflammatory activity in folk medicine by the people of Odisha.

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Conflict of interest

The authors report no conflict of interest.

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