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Preliminary studies on antiinflammatory, antipyretic, and antidiarrhoeal properties of *Evolvulus alsinoides*

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Abstract: The ethanolic extract of the whole plant of *Evolvulus alsinoides* L. (Convolvulaceae) was investigated for antiinflammatory, antipyretic and antidiarrhoeal properties in female albino rats. Preliminary phytochemical studies were carried out to confirm the presence of active chemical constituents in the plant extract, and the result showed a positive report for the presence of flavonoids, alkaloids and cardiac glycosides. The pharmacological result showed that the ethanolic extract at a dose of 250 mg/kg body weight and 500 mg/kg body weight caused a significant ($P < 0.05$) inhibition of the carrageenan-induced rat paw oedema and a significant ($P < 0.05$) reduction of hyperpyrexia induced by Brewer's yeast in rats. The extract also elicited marked antidiarrhoeal activity against castor oil-induced diarrhoea at a dose of 500 mg/kg. This study has proved scientifically the ancient claim that the whole plant of *Evolvulus alsinoides* has antiinflammatory, antipyretic and antidiarrhoeal activities.

Key words: *Evolvulus alsinoides*, antiinflammatory, antipyretic, antidiarrhoeal, rats, carrageenan, brewer's yeast, castor oil

Evolvulus alsinoides'un antiinflamatuvar, antipiretik ve antidiyare özellikleri üzerine ön çalışmalar

Özet: *Evolvulus alsinoides* L. (Convolvulaceae) tüm bitkisinin etanolik özütü, dişi albino sıçanlarda antiinflamatuvar, antipiretik ve antidiyare özellikleri bakımından araştırıldı. Bitki özütündeki aktif kimyasal bileşenlerinin varlığını doğrulamak için ön fitokimyasal çalışmalar yapıldı ve sonuç flavonoidlerin, alkaloidlerin ve kardiyak glikozitlerin varlığına dair olumlu bir rapor gösterdi. Farmakolojik sonuç, vücut ağırlığının 250 mg/kg ve 500 mg/kg dozundaki etanolik özütün sıçanlarda karajenle teşvik edilen sıçan ayak ödemi inhibisyonuna önemli ölçüde ($P < 0,05$) ve bira mayasıyla teşvik edilen hiperpireksinin azalmasına önemli ölçüde ($P < 0,05$) neden olduğunu gösterdi. Ayrıca özüt, 500 mg/kg dozda hint yağıyla teşvik edilen diyareye karşı antidiyare aktiviteyi belirgin şekilde ortaya çıkardı. Bu çalışma; antiinflamatuvar, antipiretik ve antidiyare aktiviteleri bakımından tüm *Evolvulus alsinoides* bitkisi hakkındaki atasal iddiayı bilimsel olarak kanıtlamıştır.

Anahtar sözcükler: *Evolvulus alsinoides*, antiinflamatuvar, antipiretik, antidiyare, sıçanlar, karajenan, bira mayası, hint yağı

Introduction

Evolvulus alsinoides L. (Convolvulaceae), commonly known as 'shankhpuspi' in India, Africa, and the Philippines, is an important medicinal plant employed for different ailments in India traditionally. It grows in open and grassy places throughout almost all of India and subtropical countries of the world (1,2). The oldest reports found of the use of *E. alsinoides* are from India and surrounding regions. The herb was used to treat dysentery (3). Mohammedan physicians used the plant as a general tonic to strengthen the brain and memory and to treat fever (4). *E. alsinoides* was used to treat bowel problems and to promote conception (5,6). The entire plant was considered astringent and useful for treating haemorrhages, and there are a variety of other medical applications, including use as an adaptogenic, antiphlogistic, antipyretic, antiseptic, aphrodisiac, febrifuge, stomachic, tonic, and vermifuge, in the treatment of asthma, bronchitis, scrofula, syphilis, or in "controlling night emissions," and to promote wound healing (7-10). It appears that *E. alsinoides* has some phytochemicals that are effective against the maladies for which people use them. The isolation of evolvin, kaempferol-3-O- β -D glucopyranoside, coumarin etc., from *E. alsinoides* has been previously reported (11).

Carrageenan-induced inflammation is useful in detecting orally active antiinflammatory agents (12). Oedema formation due to carrageenan in the rat paw is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The second phase of oedema is due to the release of prostaglandins, protease and lysosomes (13,14). There is evidence that compounds inhibiting carrageenan-induced oedema are also effective against cyclo oxygenase enzymes (15). Brewer's yeast-induced pyrexia has been used as a screening tool for antipyretic agents in rodents (16,17).

Castor oil causes diarrhoea due to its active metabolite, ricinoleic acid (18,19), which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandins. Non-steroidal antiinflammatory drugs (NSAIDs) are commonly used to treat inflammation and pyrexia, and opioid

derivatives are used to treat diarrhoea, but they are toxic and not always free from adverse effects (20,21). Hence a natural agent with less or no toxicity is necessary. A range of medicinal plants with antiinflammatory, antipyretic, and antidiarrhoeal properties is widely used by traditional healers. In view of *E. alsinoides*' importance in traditional medicinal systems, no substantial phytochemical and pharmacological works have been reported based on the antiinflammatory, antipyretic, and antidiarrhoeal properties. Preclinical (in vivo and in vitro) investigations have demonstrated only the anti-amnesic, anti-stress, antimicrobial, and gastroprotective activity of *E. alsinoides* (22,23). Further clinical studies are needed to prove scientifically *E. alsinoides*' antiinflammatory, antipyretic, and antidiarrhoeal properties.

In the present research, preliminary studies in female albino rats were carried out to determine the effects of ethanolic extract of *Evolvulus alsinoides* on carrageenan-induced inflammation, brewer's yeast-induced pyrexia, and castor oil-induced diarrhoea by a single experiment in each case to prove the mentioned activities.

Materials and methods

Materials

Fresh whole plants of *E. alsinoides* were collected in the Tambaram area of Chennai, Tamil Nadu (INDIA), and identified by Prof Dr. Jayaraman, Plant Anatomy and Research Centre, Tambaram, Chennai, India, where its voucher specimen (PARC/2008/152) was deposited.

Carrageenan (Sigma, US),
Indomethacin (Indocid R, Merck Sharp –Dohme),
Brewer's yeast (Hi-media, Mumbai),
Paracetamol (Cipla Ltd, Nalagarh),
Castor oil (*sd*-fine chemicals, Mumbai),
Diphenoxylate (Pfizer US pharmaceuticals group).

Preparation of plant extract

The plant materials were shade dried, powdered (800 g), and subsequently subjected to the extraction process. The solvent was removed at 30 °C using a rotary evaporator. The yield of ethanolic extract was

23 g (2.87%). Dilutions of the extract were made in 2% gum acacia for antiinflammatory, antipyretic studies and in tween 80 for antidiarrhoeal studies.

Animals

The Wistar female albino rats (180-200 g) used for this study were procured from King Institute Guindy, Chennai, India, and housed in the Institutional animal house under standard environmental conditions (23 ± 1 °C, $55 \pm 5\%$ humidity and 12 h/12 h light/dark cycle) and maintained with free access to standard diet (Hindustan Lever, Bangalore, India) and water ad libitum. The 72 animals were divided into 3 groups, each group containing 24 animals, and they were further divided into 4 sub-groups ($n = 6$ per sub-group) and housed in polypropylene cages. The protocol of animal study was approved by the Institutional Animal Ethics Committee (IAEC 03/003/08).

Phytochemical analysis

Preliminary phytochemical analysis

The preliminary phytochemical properties of the extract were tested for alkaloids with Mayers and Dragendroffs reagents, saponins glycosides with the

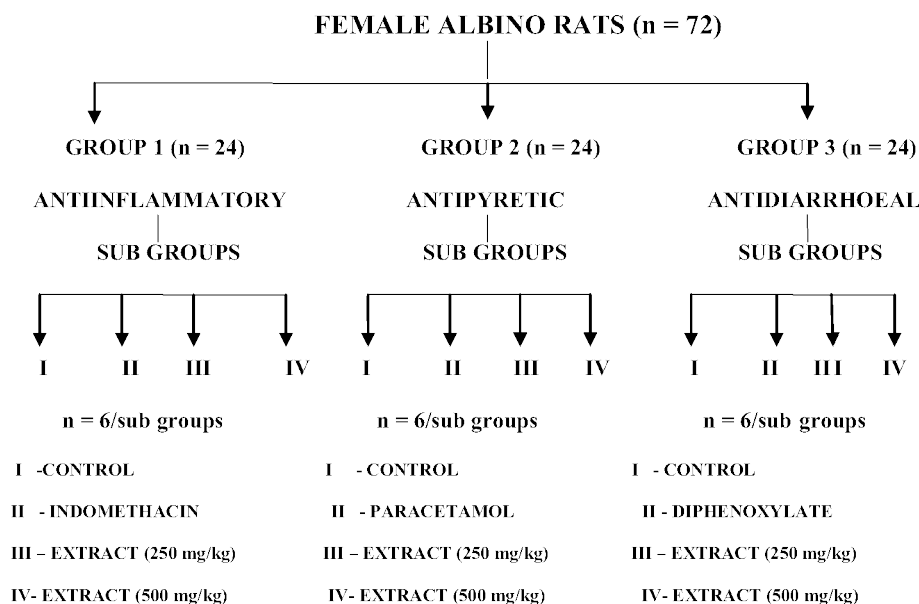
ability to produce suds, cardiac glycosides with FeCl_2 and H_2SO_4 , flavonoids with the use of Mg and HCl, anthraquinones with Borntragers reaction, terpenoids by the Liebermann-Burchard method and the use of H_2SO_4 , tannins with 1% gelatin and 10% NaCl solutions (24).

Pharmacological activities

Antiinflammatory activity

Carrageenan-induced oedema test:

Pedal inflammation in female Wistar albino rats weighing 180-200 g was produced in group 1 (25). A dose of 0.1 mL 1% carrageenan (1 g in 100 mL of gum acacia) was injected into the right hind foot of each rat under the sub plantar region. The control group of sub-group I received 2.8 mL/kg body weight of 2% gum acacia, and the indomethacin group received indomethacin in 2% gum acacia orally at a dose of 20 mg/kg body weight. The third sub-group of group I were treated with a dose of 250 mg extract/kg body weight, and the fourth sub-group of group I received 500 mg extract/kg body weight, 1 h prior to the administration of the phlogistic agent. The volume of



Schematic representation of grouping of animals for experiments

the hind paw was measured before injection of the phlogistic agent and then at hourly intervals for a period of 4 h after injection of phlogistic agent using a mercury displacement plethysmograph. A significant reduction in paw volume compared to control group was considered to be an antiinflammatory response (26,27).

$$\text{Percentage inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

V_c = paw volume for control;

V_t = paw volume for test.

Antipyretic activity

Yeast-induced hyper pyrexia in rats

Rectal temperature was recorded with a digital thermometer. Hyperpyrexia was induced in rats by injecting 20% (20 g of dried yeast in 100 mL of 2% gum acacia in normal saline) suspension of dried yeast at a volume of 20 mL/kg body weight subcutaneously (16). The animals were then fasted for the 18 h duration of the experiment, and water was provided ad libitum. In all animals of sub-groups of group 2, temperatures were taken 18 h after the yeast injection to determine the pyretic response to yeast, and rats which showed a rise in temperature of at least 0.5 °C to 1 °C were taken into the experiment. Group 2, the control group (sub-group I), received 2% gum acacia, the paracetamol group (sub-group II) received paracetamol (33 mg/kg of body weight in 2% gum acacia), and sub-groups III and IV received orally 250 mg/kg and 500 mg/kg body weight doses of the extract, respectively. The rectal temperature was recorded at 1, 2, 3, and 4 h after administration of the extract and paracetamol. Percentage reduction in rectal temperature was calculated by the following formula (28,29):

$$\% \text{ reduction} = \frac{B - C_n}{B - A} \times 100.$$

A- Normal temperature.

B- Rectal temperature at 18 h after yeast administration.

Cn- rectal temperature after drug administration.

Antidiarrhoeal activity

Castor oil-induced diarrhoea

All rats of group 3 were fasted for 18 h. Castor oil at a dose of 1 mL per animal orally was given to all sub-groups of group 3 for the induction of diarrhoea. After 30 min, the first sub group (control) of group 3 received 1 mL of 0.5% (v/v) tween 80. The second sub-group received diphenoxylate (50 mg/kg body weight) (30). The third and fourth sub-groups were treated with extract (250 mg/kg and 500 mg/kg body weight doses, respectively). Animals of all sub-groups of group 3 were placed separately in individual cages lined with filter paper (31). The filter papers were changed every hour and the severity of diarrhoea was assessed hourly for 6 h. The total number of faeces were recorded with in a period of 6 h and compared with the control group. The total number of diarrhoeal faeces of the control group was considered 100%. The results were expressed as percentage inhibition of diarrhoea (21).

Statistical analysis

The statistical analysis of data was analysed by analysis of variance (ANOVA) followed by Student t-test for comparison between experiment and control groups. The statistical package used was SPSS 15.0 software. Values were expressed as mean \pm S.E.M. and those values with $P < 0.05$ were considered significant.

Results and discussion

Antiinflammatory effect

Carrageenan-induced rat paw oedema was markedly inhibited by oral pre-treatment with the extract (250 mg/kg and 500 mg/kg body weight doses) and indomethacin (20 mg/kg body weight dose) (Table 1). In the acute inflammation model, a dose of 250 mg/kg body weight of the extract showed significant inhibition ($P < 0.05$) at 3 and 4 h, whereas indomethacin and the extract (500 mg/kg body weight) exhibited significant inhibition at 2, 3, and 4 h.

Antipyretic studies

Table 2 shows that 18 h after yeast injection, hyperthermia was recorded and continued throughout the test. The plant extract produced a reduction in hyper pyrexia induced by yeast injection in rats, with activity being pronounced within 90 min

Table 1. Effects of the ethanol extract of *Evolvulus alsinoides* whole plant on carrageenan-induced paw oedema in rats.

Group 1	Oral dose/body weight	Mean paw volume (in mL)				
		Before carrageenan	After carrageenan			
			1 h	2 h	3 h	4 h
I Control 2% gum acacia	2.8 mL/kg	1.67 ± 0.12	2.27 ± 0.6	3.28 ± 0.09	4.23 ± 0.04	4.67 ± 0.04
II Indomethacin	20 mg/kg	1.84 ± 0.06	2.12 ± 0.07	1.98 ± 0.05 *	1.87 ± 0.06*	1.85 ± 0.07*
III <i>E. alsinoides</i> extract	250 mg/kg	1.9 ± 0.07	2.25 ± 0.04	2.94 ± 0.05	2.23 ± 0.02*	1.95 ± 0.05*
IV <i>E. alsinoides</i> extract	500 mg/kg	1.93 ± 0.08	2.18 ± 0.02	2.00 ± 0.02*	1.99 ± 0.06*	1.9 ± 0.05*

All values are expressed in mean ± SEM (n = 6)

*P < 0.05 significant compared to control

after administration of the extract. Also, within 2 h of administration of the extract, the plant extract was as effective as paracetamol in reducing hyperthermia (P < 0.05).

Antidiarrhoeal studies

The rats of sub-group I of group 3 that did not receive the plant extract showed typical diarrhoea signs such as watery and frequent defecation. The extract at a dose of 250 mg/kg did not show any significant reduction in castor oil-induced diarrhoea, but showed significant reduction (P < 0.001) at a dose of 500 mg/kg body weight. Both doses of the extract significantly

decreased (P < 0.05) diarrhoea as compared to the control group (Table 3), but not very significantly when compared with the diphenoxylate group.

Phytochemical studies

Phytochemical studies showed that the applied extract was positive for alkaloids, cardiac glycosides, and flavonoids, and negative for saponins and terpenoids.

In the present study, the pharmacological activities of the ethanol extract of *E. alsinoides* were studied. The extract was found to significantly inhibit

Table 2. Effects of the ethanol extract of *E. alsinoides* on yeast-induced pyrexia in rats.

Group 2	Dose/kg body weight	Rectal temperature (°C)		Rectal temperature after drug administration in °C (percentage inhibition in rectal temperature)			
		Normal (A)	After yeast + 18 h (B)	+ 1 h (C1)	+ 2 h (C2)	+ 3 h (C3)	+ 4 h (C4)
Control 2% gum acacia (I)	2.8 mL/rat	37.4 ± 0.08	38.8 ± 0.23	38.7 ± 0.1 (---)	38.6 ± 0.13 (---)	38.7 ± 0.08 (---)	38.7 ± 0.08 (---)
Paracetamol (II)	33 mg	37.5 ± 0.13	38.6 ± 0.12	37.6 ± 0.11* (90)	37.6 ± 0.09* (90)	37.6 ± 0.11* (90)	37.5 ± 0.13* (100)
Extract (III)	250 mg	37.31 ± 0.08	38.7 ± 0.05	38.3 ± 0.16 (28.77)	37.7 ± 0.07* (71.94)	37.6 ± 0.07* (79.31)	37.5 ± 0.04* (86.33)
Extract (IV)	500 mg	37.4 ± 0.06	38.7 ± 0.08	38.1 ± 0.08* (46.15)	37.6 ± 0.05* (84.61)	37.6 ± 0.05* (84.61)	37.5 ± 0.05* (92.30)

Effects of the ethanol extract of *E. alsinoides* on yeast-induced pyrexia in rats.

All values are expressed in mean ± SEM (n = 6), percentage reduction in rectal temperature is given within parentheses

*P < 0.05 significant compared to control

Table 3. Effects of the ethanol extract of *E. alsinoides* on castor oil-induced diarrhoea in rats.

Group 3	Dose	Total number of faeces	Total number of diarrhoeal faeces	Inhibition (%)	Total weight of faeces	Inhibition (%)
Control (I)	Castor oil 1 mL + 0.5% tween 80	25.2 ± 1.49	18 ± 0.71	0.00	9.76 ± 0.35	0.00
Diphenoxylate (II)	50 mg/kg + castor oil (1 mL)	7.8 ± 0.37***	5.2 ± 1.30***	71.72	1.76 ± 0.57***	81.97
Extract (III)	250 mg/kg + castor oil (1 mL)	18.65 ± 0.98*	6.3 ± 0.84	65	4.78 ± 1.28*	51.29
Extract (IV)	500 mg/kg + castor oil (1 mL)	18 ± 0.71*	5.9 ± 1.30***	67.23	2.98 ± 0.58**	69.46

Values are expressed as mean ± SEM (n = 6)

P < 0.01**, P < 0.001*** when compared with control.

carrageenan-induced rat paw oedema, a test which has a significant predictive value for antiinflammatory agents acting by inhibiting the mediators of acute inflammation (32). The antiinflammatory effect of the crude extract of the plant at a 500 mg/kg dose in the second hour after carrageenan injection strongly suggests its NSAID-like activity (Table 1). Similarly, the standard drug indomethacin produced a significant antiedematous effect, which was in accordance with previous reports indicating that NSAIDs show marked inhibition of carrageenan-induced oedema in rats (33,34). The extract showed antipyretic activity in rats made hyperthermic by yeast injection. The active constituents may exhibit both central and peripheral actions because pyrexia and inflammation are central and peripheral processes, respectively. *E. alsinoides* extract produced significant antipyretic activity at a dose of 500 mg/kg (Table 2). Since antipyretic activity is commonly mentioned as characteristics of drugs or compounds which have an inhibitory effect on prostaglandin (PG) biosynthesis, yeast-induced hyperpyrexia in a rat model was employed (35). In general, NSAIDs produce their antipyretic action through inhibition of prostaglandin synthetase within the hypothalamus (36,37). Although there is no direct evidence that the extract of *E. alsinoides* interferes with PG synthesis in hypothalamus, it can only be supported by a related study in which *E. alsinoides* extract was found to have antistress activity, immunomodulatory and

gastroprotective activity, and so the action of extract particularly on PG synthesis requires further confirmation (11,22). It appears that antipyretic action of *E. alsinoides* extract may be related to the inhibition of PG synthesis in the hypothalamus. Such reduction of the rectal temperature of the tested animals by extract appears to be due to the presence of a single bio-active substance or mixture of compounds in them. Thus the activities shown by the crude extract could be a useful factor in its application in febrifuge. In this study, ethanol extract of *E. alsinoides* exhibited a significant antidiarrhoeal activity (Table 3) when compared with the control group. The extract of *E. alsinoides* showed dose-dependent effects in controlling the diarrhoea. The results are similar to those of the standard drug diphenoxylate with regard to severity of diarrhoea (38). These findings support the use of *E. alsinoides* in various ailments. Therefore, further studies are needed for the isolation and characterisation of the active constituents responsible for these activities.

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