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Analgesic, diuretic, and anti-inflammatory principle from *Scoparia dulcis*

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Scoparinol, a diterpene, isolated from *Scoparia dulcis* showed significant analgesic ($p < 0.001$) and anti-inflammatory activity ($p < 0.01$) in animals. A sedative action of scoparinol was demonstrated by a marked potentiation of pentobarbital-induced sedation with a significant effect on both onset and duration of sleep ($p < 0.05$). Measurement of urine volume after administration of scoparinol indicated its significant diuretic action.

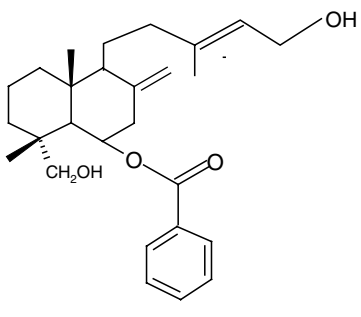
1. Introduction

Scoparia dulcis (Scrophulariaceae) is an erect or ascending annual leafy herb grown mostly in Bangladesh, India, Bhutan, and the Tropics of America and sporadically in Africa and Australia. An infusion of the leaves is used in fever, cough and bronchitis and as a gargle for toothache. A hot infusion is used as a diuretic and a cold decoction of the plant is used against gravel and kidney complaints. An infusion of roots, leaves and stems is useful in diarrhea and dysentery [1]. Previous phytochemical investigations on *Scoparia dulcis* revealed the presence of a variety of secondary metabolites like terpenoids, flavonoids, steroids, glycosides and quinones. In one phytochemical investigation the presence of stearic, myristic and linolic acid, dulciol, scoparol and mannitol was reported [2]. Other investigations on this plant afforded dulcinol, β-sitosterol, dulciolene, friedelin, glutinol, betulinic acid, dulcinoic acid, scoparic acid A, B and C, scopadulin, scopadulcic acid A and B [3 –6]. In continuation of our studies on medicinal plants available in Bangladesh for their chemical constituents and biological activities we isolated two new diterpenes namely scoparinol and dulcinol closely related to scoparic acid A and scopadulcic acid from the whole plant extract of *Scoparia dulcis* [7]. In the present paper we report the effect of scoparinol on analgesic, diuretic and anti-inflammatory activities in animal models.

2. Investigations, results and discussion

The experimental findings from the Hippocratic screening test [8] showed that the animals behaved passively in response to head tap and fearfully in response to body grasp. The results also indicated a decrease in motor activity, loss of corneal reflex, presence of writhing movement, marked urination and slight defecation. To find the effect of the drug on the CNS, the spontaneous motor activity test has been designed [9]. The results were statistically significant and showed that the spontaneous motor activity of the treated animals was decreased gradually. As scoparinol reduced motor activity and many central depressants act on the cerebral cortex, the chance of scoparinol having an effect on the cerebral cortex was evaluated by the pentobarbital-induced sleeping time test [10]. From the experimental results, it was evident that scoparinol caused a marked potentiation in both onset and duration of pentobarbital-induced sedation (Fig. 1). If the potentiation of drug-induced sedation, as observed from the pentobarbital-induced sleeping time test, was due to blocking of H_1 receptors, scoparinol should either prevent or reduce the degree of inflammation. One of the common features of inflammation irrespective of cause is the genesis of edema. On the basis of this the next experiment was designed to investigate the effect of scoparinol on carragenin-induced edema [11]. The result of this experiment showed that the experimental compound scoparinol reduced the carragenin-induced swelling of the rat paw. Percent inhibition of edema was approximately 25% at 24 h after scoparinol administration at 100-mg/kg-body weight (Fig. 2).

The experiment on analgesia aimed to investigate whether or not scoparinol possesses any analgesic activity. This is because all classical NSAID act as potent analgesics. The experimental results from the hot plate test [12] suggested that scoparinol like the classical NSAID possesses marked analgesic activity which was statistically significant at the 0.1% ($P < 0.001$) level (Fig. 3). Again the standard drug paracetamol was compared with the test compound and a significant correlation was obtained between effects of the test compound and the standard drug. Inflammation, a dy-



scoparinol

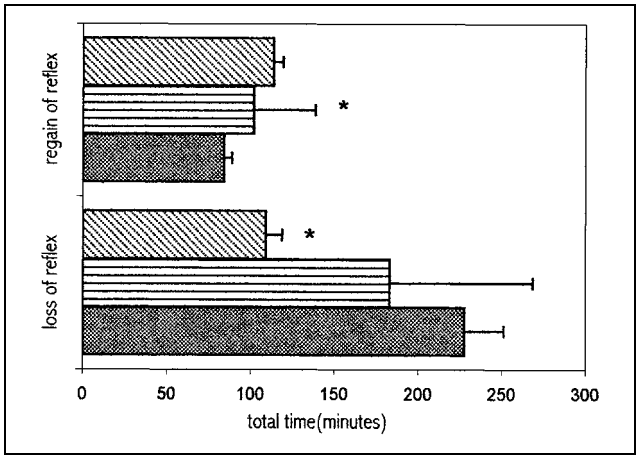


Fig. 1: Effect of scoparinol on pentobarbital induced sleeping time in mice. Each bar represents the mean ±SE. The sleeping time in controls was taken as 100%. * $p < 0.05$. scoparinol (30 mg/kg); scoparinol (10 mg/kg); control

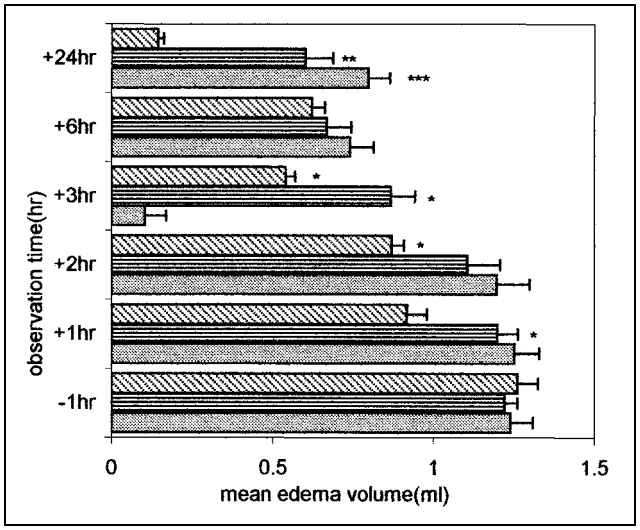


Fig. 2: Effect of scoparinol on carragenin induced edema. Each bar represents mean ±SE. The volume of edema in controls was taken as 100%. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ▨ positive control (10 mg/kg); ▤ scoparinol (100 mg/kg); ■ control

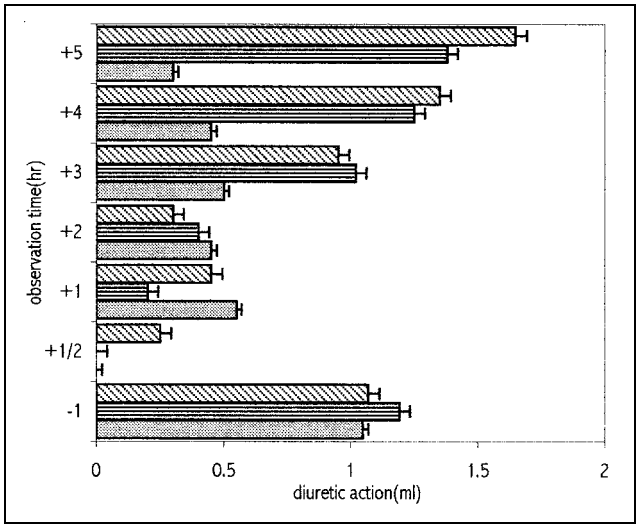


Fig. 4: Effect of scoparinol on urine volume. Each bar represents mean diuretic action ±SE. The diuretic action of the control was taken as zero. ▨ scoparinol (30 mg/kg); ▤ scoparinol (10 mg/kg); ■ control

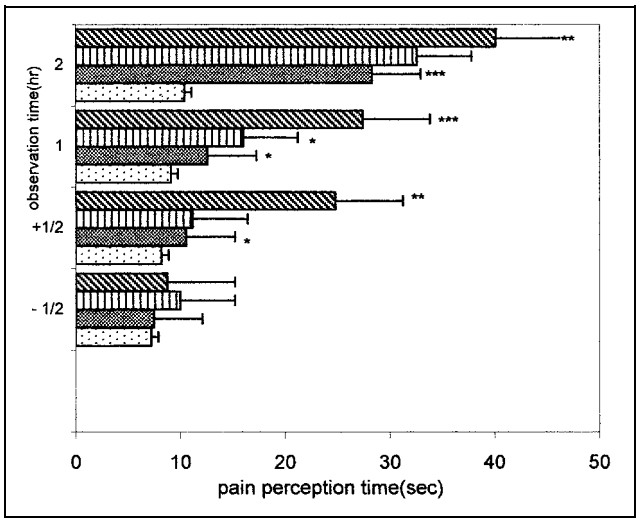


Fig. 3: Effect of scoparinol on pain perception in mice. Each bar represents mean ±SE. The pain perception time in controls was taken as 100%. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. ▨ paracetamol (5 mg/kg); ▤ scoparinol (30 mg/kg); ▥ scoparinol (10 mg/kg); ■ control

namic process considered as a protective mechanism, leads to a chronic inflammatory state when deregulated. Again the mechanism of drug-induced sedation may be due to CNS depression by GABA inhibition or may be due to blocking of H_1 receptors. If the mechanism of the potentiation of drug-induced sedation is due to antihistaminic effect of a drug, scoparinol should either prevent or reduce the degree of inflammation. During the condition of inflammation associated with pain and fever, arachidonic acid is liberated from the phospholipid fraction of cell membranes, and then enzymatically transformed to prostaglandins which sensitize blood vessels to the effect of mediators that increase permeability, such as bradykinin, 5-HT and histamines. One of the common features of inflammation irrespective of cause is the genesis of edema. It is believed that the edema test is the most prominent model for testing the anti-inflammatory activity of a drug [11]. The result of this experiment suggested that scoparinol reduces the carragenin induced swelling of the rat paw. The result was statistically significant at the 1% level. Again the reduction of carragenin-induced edema after scoparinol administration was compared with the standard drug phenylbutazone. After the administration of phenylbutazone, the decrease in edema volume was most prominent in the third hour. Inhibition of edema was approximately 25%, 24 h after the administration of the test compound at 100 mg/kg.

As the result of the Hippocratic screening test indicated that scoparinol caused marked urination, urine volume was measured. The data obtained from the test revealed that the compound increased urine volume in a dose dependent manner. The diuretic action of scoparinol was prominent 4 h after administration (Fig. 4). 6 h after administration, the diuretic action was found to be slightly reduced, perhaps due to a decrease in drug concentration in the experimental animals.

Though scoparinol appeared to cause marked diuresis, the actual mode of action, which may be due to an increase in electrolyte loss (Na^+, K^+Cl^-) resulting from its effect on loop permeability, or a reduction of ADH secretion or an inhibitory effect on carbonic anhydrase, is not clear.

This result supports the findings of the anti-inflammatory test. Since edema is characterized by increased fluid retention, the reduction in carragenin induced edema may be due to this diuretic effect of scoparinol.

3. Experimental

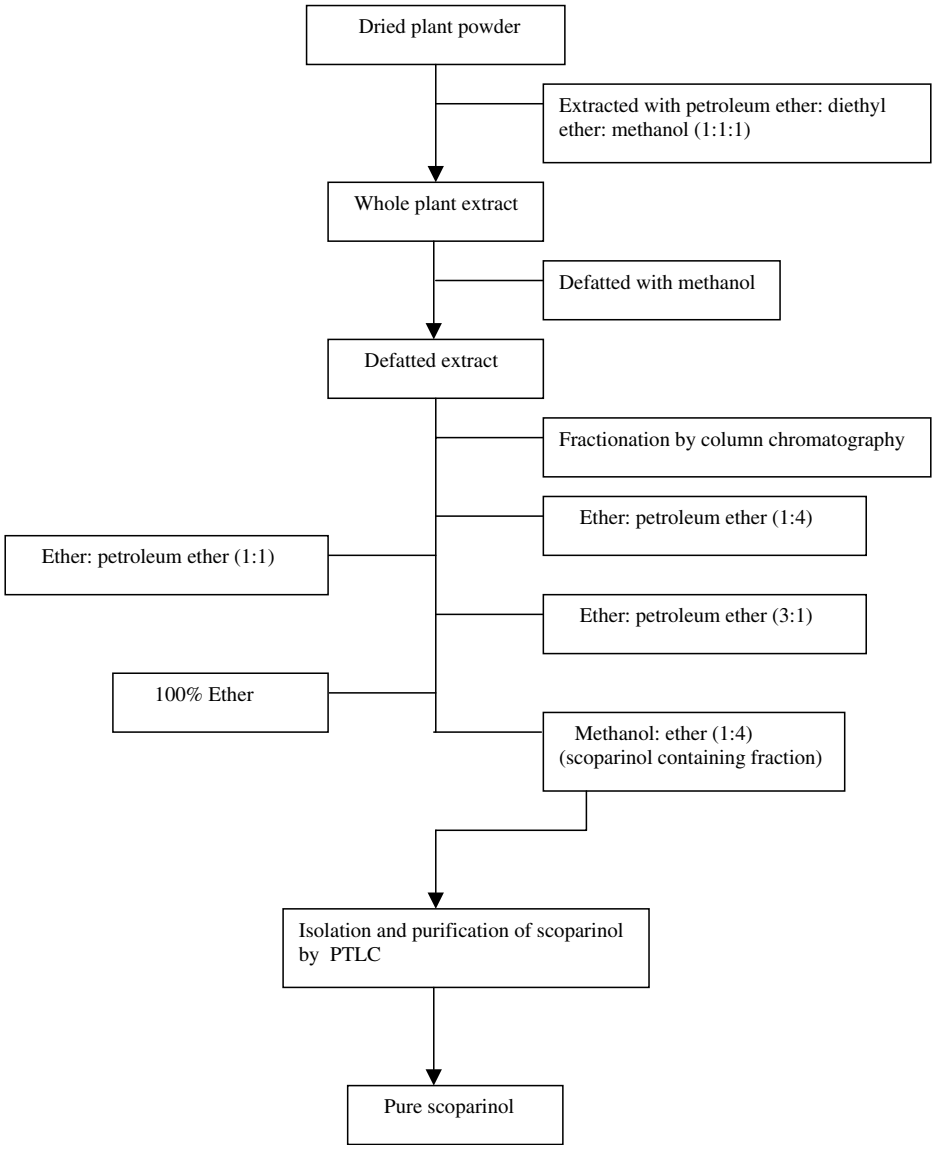
3.1. Chemistry

The mature plant *Scoparia dulcis* was collected from Savar, Dhaka and identified by the National Herbarium of Bangladesh. The sundried plant was ground to coarse powder. The coarse powder (6 kg) of the sundried plant was extracted with a mixture of solvents comprising petroleum ether, diethyl ether and methanol in equal proportions. The extract was evaporated to dryness and defatted. The resulting extract was separated by CC (silica gel) using petroleum ether, diethyl ether and methanol with increasing polarity. The fraction thus obtained from CC was subsequently separated and purified by repeated TLC to give 300 mg scoparinol. The pure scoparinol was characterized and identified by comparing its Rf value and spectral data with those of the reference compound. The complete isolation method is shown in the Scheme.

3.2. Pharmacology

Swiss albino mice weighing between 20–30 g were employed as experimental animals. Long Evan rats weighing between 150–170 g were used for anti-inflammatory tests and acute and sub-acute toxicity studies. The experimental animals were randomly divided into different groups on the

Scheme



basis of the number of samples and doses to be applied with 5 mice in each group. All the mice and rats were individually weighed and the dose of the test samples and control materials adjusted accordingly. The animals were kept at room temperature under conditions of a natural light and dark schedule. The animals were kept for at least 7 to 10 days in the animal house to become adapted to the environment before being employed in the experiment.

The test samples for administration in the experiment were prepared as a suspension with a few drops of Tween®-20 (1%) as a suspending agent.

3.2.1. Pentobarbital induced sleeping time

The potentiating or inhibiting effect of scoparinol on drug-induced sedation was determined by the pentobarbital-induced sleeping time method [13].

In this method, the test compound scoparinol was given intraperitoneally to the animals of groups I and II at a dose of 10 and 30 mg/kg body wt. respectively 30 min before the administration of pentobarbital (3mg/kg body wt.), while the animals of the control group C were supplied with saline water containing Tween 20. The onset of sleep and total sleeping time were the time taken for the loss of righting reflex and the time between loss and regain of righting reflex respectively. The onset of sleep and total sleeping time were recorded for both control and experimental groups.

3.2.2. Analgesic activity

The objective of the study was to observe the effect of scoparinol on pain perception of the animals. This was determined by the hot plate method [14]. In this experiment paracetamol was used as the standard drug at a dose of 5 mg/kg-body weight. The test samples were injected intraperitoneally to the mice of the experimental groups at doses of 10 and 30 mg/kg body wt. respectively while the animals of the control group were given only saline water with Tween 20. The animals of all groups were then placed on a hot plate, with temperature maintained at 55 ± 5 °C. The reaction time was the time between placement of the animals on the hot plate and licking of the fore and/or hind paw. A cut off time of 40 seconds was allowed so as to avoid any injury to the paws due to heat. Increase or decrease of the reaction time was noted for control as well as treated groups at different time intervals.

3.2.3. Diuretic activity

The animals in groups of three were put in the metabolic cages and after an initial adjustment period of 3 days, the test substance was administered intraperitoneally to the animals of the experimental group at doses of 10 and 30 mg/kg body wt. The effect of scoparinol at different doses was recorded by measurement of urine volumes of each group. The diuretic action was calculated using the following formula:

Diuretic action = urine volume of sample/urine volume of control

3.2.4. Anti-inflammatory activity

Screening for anti-inflammatory activity of scoparinol was done with a carragenin induced paw edema model [15, 16]. The rats were used after acclimatization for 8 days to the laboratory conditions. The initial paw volumes were determined up to a fixed mark by mercury displacement

techniques with a travelling microscope [16]. Phenylbutazone and scoparinol dissolved in normal saline were administered intraperitoneally to the animals of the two experimental groups at doses of 10 and 100 mg/kg body wt. respectively 1 h before the administration of carragenin (0.1 ml of 1% solution in normal saline) into the planter surface of the right hind paw. The animals of the control group were given only saline water containing Tween-20. The paw volumes were measured at hourly intervals. Percent inhibition of paw edema was determined using the following formula:

$$\% \text{ Inhibition} = \frac{V_0 - V_1}{V_0} \times 100$$

where V_0 and V_1 are average edema volumes of the control and treated groups respectively.

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