

In vivo analgesic activity of ethanolic extracts of two medicinal plants - *Scoparia dulcis* L. and *Ficus racemosa* Linn.

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Abstract

Fruits and whole herb of two medicinal plants, *Ficus racemosa* Linn. (Moraceae) and *Scoparia dulcis* L. (Scrophulariaceae) were extracted in 95% ethanol to evaluate for centrally acting analgesic potential using hot plate and peripheral pharmacological actions using acetic acid induced writhing test in mice. The crude extracts of both the plants were found to have significant ($p < 0.001$) analgesic activity at the oral dose of 100 & 200 mg/kg b. wt., in the tested models. In hot plate test *S. dulcis* showed increased latency period than *F. racemosa* whereas in acetic acid induced writhing test *F. racemosa* showed reduced number of writhes than *S. dulcis* at two dose levels which are significant ($p < 0.001$) compared to control. The results obtained support the use of fruits of *F. racemosa* and whole herb of *S. dulcis* in painful conditions acting both centrally and peripherally.

Keywords: Analgesic; *Ficus racemosa* Linn.; Mice; Moraceae; *Scoparia dulcis* L.; Scrophulariaceae.

Introduction

Due to having adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant-based drugs (Kumara, 2001).

Scoparia dulcis L. belongs to the family Scrophulariaceae is commonly known as Sweet Broomweed, has been used as a remedy for diabetes mellitus in India (Satyanarayana, 1969). It is a perennial herb that is distributed widely in tropical and subtropical regions where the fresh or dried plant has traditionally been used as one of remedies for stomach troubles, hypertension, diabetes, inflammation, bronchitis, hemorrhoids and hepatitis and as an analgesic and antipyretic (Freire *et al.*, 1993; Hayashi *et al.*, 1993). Isolation of different chemical compounds including Scoparic acid A, Scoparic acid B, Scopadulcic acid A and B, Scopadulciol and Scopadulin has been

reported previously (Hayashi *et al.*, 1990; Hayashi *et al.*, 1991; Hayashi *et al.*, 1993). In addition, these compounds have been identified as contributor to the observed medicinal effect of this plant. These compounds were found to possess various biological activities such as inhibitor against replication of herpes simplex virus, gastric H⁺, K⁺ ATPase activator and antitumor promoting activity etc. (Hayashi *et al.*, 1991). Besides glutinol (Freire *et al.*, 1991, 1993) and scoparinol (Ahmed *et al.*, 2001), isolated from the plant extract were found to exert significant analgesic activity.

Ficus racemosa Linn. Syn. *Ficus glomerata* Roxb belonging to the family Moraceae is commonly known as 'Jagya-dumur' (Bengali), 'Gular' (Hindi) and 'Udumbara' (Sanskrit) is a well-known moderate sized to large spreading tree with ovate, ovate-lanceolate leaves. The leaves are used in dysentery, diarrhoea, bilious affection and in dysmenorrhoea; barks and fruits are also used in dysentery, diarrhoea and in diabetes (Chopra *et al.*, 1958; Kirtikar and Basu, 1975; Nadkarni *et al.*, 1976). The anti-diarrhoeal activity (Mandal *et al.*, 1997a), hypoglycaemic activity (Mandal *et al.*, 1997b), anti-inflammatory activity (Mandal *et al.*, 1998a), hepatoprotective activity (Mandal *et al.*, 1998b, 1999) and the treatment of bronchitis (Mandal *et al.*, 2000) are properties

of the leaf extract of *F. racemosa* have been reported (Subhash C *et al.*, 2000). The alcoholic extract of the stem bark of the plant possessed anti-protozoal activity against *Entamoeba histolytica* and its use in the treatment of mumps, smallpox, haematuria and inflammatory conditions were reported by Mandal *et al.* (2000). The ethanolic extract of bark and fruit was reported to have significant analgesic activity (Ferdous *et al.*, 2008). Its fruits are effective against leprosy, diseases of the blood, fatigue, bleeding nose and cough and roots are used as a medicine against hydrophobia (Mandal *et al.*, 2000). The present study was undertaken to evaluate the analgesic activity of fruits of *F. racemosa* and whole herb of *S. dulcis* in mice using two experimental pain models.

Materials and Methods

Drugs and chemicals

Acetic acid was obtained from Merck, Germany. Tween-80 was obtained from BDH Chemicals, UK. Normal saline solution was purchased from Beximco Infusion Ltd., Bangladesh. Diclofenac sodium was obtained from Square Pharmaceuticals Ltd., Bangladesh.

Plant material

For this present investigation, the *S. dulcis* and *F. racemosa* were collected from Village: Milua, Thana: Doulatpur, District: Manikgong, Bangladesh in July 2008 and were identified at the Bangladesh National Herbarium, Mirpur, Dhaka where the Voucher specimen no: 32766 and 32767 for *Scoparia dulcis* L. and *Ficus racemosa* Linn. respectively were deposited. The collected plant parts were dried for one week and ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of the extract

About 180 gm of powdered material was taken in a clean, flat bottomed glass container and soaked in 500 ml of 95% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK). The filtrate (ethanol extract) obtained was evaporated

using rotary evaporator. It rendered a gummy concentrate of reddish black color. The gummy concentrate was designated as crude extract of ethanol. The extract was transferred to a closed container for further use and protection.

Animals

Young Swiss-albino mice of either sex aged 4-5 weeks, average weight 20-25 gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDRDB). They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed ICDDRDB formulated rodent food and water *ad libitum*. The set of rules followed for animal experiment were approved by the institutional animal ethical committee (Zimmermann, 1983).

Phytochemical screening

Phytochemical screening of the prepared extracts was conducted with various qualitative tests to identify the presence of chemical constituents. To perform the tests the following chemicals and reagents were used: Carbohydrates with Molisch's test, glycoside with water and sodium hydroxide solution, saponins with the capability of producing suds, steroids with chloroform and sulphuric acid, flavonoids with Mg and HCl, tannins with ferric chloride solution, gum with Molish reagents and concentrated sulfuric acid. Alkaloids were tested with Mayer's reagent, Hager's reagent and Dagendorff's reagent. These were identified by characteristic color changes using standard procedures (Ghani, 2003).

Analgesic activity

Hot plate method

Experimental animals of either sex were randomly selected and divided into four groups designated as group-I, group-II, group-III and group-IV consisting of five mice in each group for control, positive control and test sample group respectively. Each group received a particular treatment i.e. control (1% Tween-80 solution in water, 10ml/kg, p.o.), positive control (Diclofenac sodium 10 mg/kg, p.o.) and the test sample (ethanolic extract of 100 mg/kg, p.o. & 200 mg/kg, p.o. respectively). The animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5 °C. A cut off period of 15 s (Franzotti *et al.*, 2000) was observed to avoid damage to the paw.

Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after oral administration of the samples (Eddy *et al.*, 1953; Kulkarni, 1999; Toma *et al.*, 2003).

Acetic acid induced writhing method

The analgesic activity of the samples was evaluated using acetic acid induced writhing method in mice (Ahmed *et al.*, 2004). In this method, acetic acid is administered intraperitoneally to the experimental animals to create pain sensation. As a positive control, any standard NSAID drug can be used. In the present study Diclofenac sodium was used to serve the purpose. The plant extract was administered orally in two different doses (100 and 200 mg/kg body weight) to the Swiss Albino mice after an overnight fast. Test samples and vehicle were administered orally 30 minutes prior to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1ml/10g) but Diclofenac sodium was administered 15 minutes prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse of all groups were observed individually for counting the number of writhing they made in 15 minutes commencing just 5 minutes after the intraperitoneal administration of acetic acid solution. Full writhing was not always

accomplished by the animal, because sometimes the animals started to give writhing but they did not complete it. This incomplete writhing was considered as half-writhing. Accordingly, two half-writhing were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group while Diclofenac sodium (10 mg/kg) was used as a reference substance (positive control).

Statistical analysis

The results of statistical analysis for animal experiment were expressed as mean \pm SEM and were evaluated by ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. The $p < 0.05$, 0.001 were considered to be statistically significant.

Results

Phytochemical Screening

The results of phytochemical screening are given in Table 1. Phytochemical analysis of the plant extracts revealed the presence of alkaloids, carbohydrates, glycoside, tannis in *S. dulcis* and tannins, gums, flavonoids, alkaloids in *F. racemosa*.

Table 1: Results of chemical group test of the ethanolic extracts of *S. dulcis* & *F. racemosa*.

Extract	Steroid	Alkaloid	Tannin	Carbohydrate	Gum	Glycoside	Flavonoid	Saponin
EESD	-	++	++	++	-	++	-	-
EEFR	+	+	++	-	+		+++	-

EESD: Ethanolic extracts of *Scoparia dulcis* L.; EEFR: Ethanolic extracts of *Ficus racemosa* Linn. ; (+): Present; (-): Absent

Hot plate method

Results of hotplate test are presented in Table 2 and Table 3 for the crude extracts of *S. dulcis* and *F. racemosa* respectively. The extracts of both the plants were found to exhibit a dose dependent increase in latency time when compared with control. At 90

minutes, the percent inhibition of two different doses (100 and 200 mg/kg body weight) was 54.78% & 62.22% for *S. dulcis* and 50.14% & 56.56% for *F. racemosa* respectively. The results were found to be statistically significant ($p < 0.001$).

Table 2: Effect of ethanolic extracts of *S. dulcis* on latency to hot plate test in mice.

Groups	Dose (mg/kg)	Mean latency (s) before and after drug administration(s)				% inhibition		
		0 min	30 min	60 min	90 min	30 min	60 min	90 min
Group-I	Vehicle	2.30± 0.202	2.50± 0.187	2.44± 0.219	2.32± 0.116	-	-	-
Group-II	10	1.96± 0.217	4.56± 0.281**	8.92± 0.860**	11.53± 1.159**	45.15	72.68	79.89
Group-III	100	2.01± 0.162	3.86± 0.551**	5.82± 0.670**	5.13± 0.287**	35.23	58.14	54.78
Group-IV	200	2.12± 0.177	4.39± 0.692**	6.71± 0.529**	6.14± 0.605**	43.08	63.64	62.22

Results are presented as mean ± SEM, (n=5), *: p<0.05, **: p<0.001 dunnet test as compared to control.

Table 3: Effect of ethanolic extracts of *F. racemosa* on latency to hotplate test in mice.

Groups	Dose (mg/kg)	Mean latency (s) before and after drug administration				% inhibition		
		0 min	30 min	60 min	90 min	30 min	60 min	90 min
Group-I	Vehicle	2.26 ± 0.219	2.45± 0.225	2.16± 0.197	2.58± 0.261	-	-	-
Group-II	10	2.34± 0.088	5.62± 0.624**	7.97± 0.649**	11.67± 1.007**	56.47	72.86	77.91
Group-III	100	2.21± 0.073	3.27± 0.264**	5.19± 0.777**	5.17± 0.619**	25.18	58.29	50.14
Group-IV	200	2.07± 0.217	3.57± 0.850**	6.34± 0.517**	5.93± 0.460**	31.50	65.89	56.56

Results are presented as mean ± SEM, (n=5), *: p<0.05, **: p<0.001 dunnet test as compared to control.

Acetic acid-induced writhing test

Table 4 and Table 5 show the effects of the extracts of *S. dulcis* and *F. racemosa* respectively on acetic acid-induced writhing in mice. Both doses of extracts of *S. dulcis* & *F. racemosa* showed significant reduction (p<0.001) of writhing induced by the acetic acid after oral administration in a dose

dependant manner. After oral administration of two different doses (100 and 200 mg/kg body weight), the percent inhibition was 39.61% & 53.06% for *S. dulcis* and 42.08% & 59.90% for *F. racemosa* respectively. The reference drug diclofenac sodium was found more potent than both the plant extracts at all of the dose levels.

Table 4: Effect of ethanolic extracts of *S. dulcis* on acetic acid induced writhing in mice.

Groups	Treatment	Dose, route	No. of writhing	Percent inhibition
Group-I	1% Tween 80 in water	0.4 ml/mouse, p.o	40.90 ± 1.28	--
Group-II	Diclofenac Sodium	10 ml/kg, p.o	9.5 ± 1.031**	76.77
Group-III	<i>S. dulcis</i>	100 mg/kg, p.o	24.70 ± 1.50**	39.61
Group-IV	<i>S. dulcis</i>	200 mg/kg, p.o	19.2 ± 1.35**	53.06

Each value represents the mean ± SEM of 5 experiments. *: p<0.05, **: p<0.001 dunnet test as compared to control.

Table 5: Effect of ethanolic extracts of *F. racemosa* on acetic acid induced writhing in mice.

Groups	Treatment	Dose, route	No. of writhing	Percent inhibition
Group-I	1% Tween 80 in water	0.4 ml/mouse, p.o	40.40 ± 1.44	--
Group-II	Diclofenac Sodium	10 ml/kg, p.o	10.00 ± 0.791**	75.25
Group-III	<i>F. racemosa</i>	100 mg/kg, p.o	23.40 ± 1.71**	42.08
Group-IV	<i>F. racemosa</i>	200 mg/kg, p.o	16.20 ± 1.40**	59.90

Each value represents the mean ± SEM of 5 experiments. *: p<0.05, **: p<0.001 dunnet test as compared to control.

Discussion

Acetic acid induced writhing in mice attributed visceral pain finds much attention of screening analgesic drugs (Hasan *et al.*, 2010). The crude extracts of both the plants showed significant analgesic action compared to the reference drug diclofenac sodium but *F. racemosa* was found to exhibit higher analgesic activity than *S. dulcis* against acetic acid induced pain in mice at two dose levels i.e. 100 & 200 mg/kg b. wt.

Pain sensation in acetic acid induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid (Ahmed *et al.*, 2006) via cyclooxygenase (COX), and prostaglandin biosynthesis (Duarte *et al.*, 1988). In other words, the acetic acid induced writhing has been associated with increased level of PGE₂ and PGF_{2α} in peritoneal fluids as well as lipoxygenase products (Derardt *et al.*, 1980). The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability (Zakaria *et al.*, 2008). The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing will render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Duarte *et al.*, 1988; Ferdous *et al.*, 2008). The significant pain reduction of both the plant extracts might be due to the presence of analgesic principles acting with the prostaglandin pathways. It was found that the observed analgesia in *S. dulcis* was demonstrated by the active constituents, Glutoniol, a triterpene (Freire *et al.*, 1991, 1993) and Scoparinol, a diterpene (Ahmed *et al.*, 2001) isolated from the plant extract through a peripherally acting mechanism similar to the non-steroidal anti-inflammatory agents, such as indomethacin and diclofenac sodium. The abdominal writhing induced by acetic acid was also reported to be less selective (Collier *et al.*, 1968) and proposed to act indirectly by releasing endogenous mediators stimulating

neurons that are sensitive to other drugs such as narcotics and centrally acting agents (Toma *et al.*, 2003).

Preliminary qualitative phytochemical screening reveals the presence of alkaloids, carbohydrates, glycosides & tannis in *S. dulcis* and tannins, gums, flavonoids & alkaloids in *F. racemosa*. Therefore, it is assumed that these compounds may be responsible for the observed analgesic activity. Flavonoids were reported to have a role in analgesic activity primarily by targeting prostaglandins (Rajnarayana *et al.* 2001; Rao *et al.*, 1998). There are also reports on the role of tannins in anti-nociceptive activity (Vanu *et al.*, 2006). Besides alkaloids are well known for their ability to inhibit pain perception (Uche *et al.*, 2008).

The extracts of the plants and diclofenac sodium (10 mg/kg) also presented a longer latency time than the control group in the hot plate test in a dose related manner. At 90 minutes & 200 mg/kg, p.o. administration of the plant extracts the percent inhibition was found 62.22% & 56.56% for *S. dulcis* and *F. racemosa* respectively. The hot plat method is considered to be selective for the drugs acting centrally. The hot plat test measures the complex response to a non-inflammatory, acute nociceptive input and is one of the models normally used for studying central nociceptive activity (Sabina *et al.*, 2009). It is an established fact that any agent that causes a prolongation of the hot plate latency using this test must be acting centrally (Ibironke and Ajiboye, 2007). Therefore, the ethanolic extracts of the plants must have a central activity. Again, narcotic analgesics inhibit both peripheral and central mechanism of pain, while NSAIDs inhibit only peripheral pain (Elisabetsky *et al.*, 1995; Pal *et al.*, 1999). The plant extracts of *S. dulcis* and *F. racemosa* exhibited both types of pain inhibition. The analgesic effect of the plants in both models suggests that they have been acting through central and peripheral mechanism (Sabina *et al.*, 2009).

Conclusion

In conclusion, we can confirm that the ethanolic extracts of *S. dulcis* whole herb and *F. racemosa* fruits are endowed with both central and peripheral analgesic properties. However, further study is needed in order to understand the precise mechanism. In future experiments, studies with purified fractions of the extract can be conducted for further pharmacological and toxicological characterization, such as the research of the mechanisms involved in the central and peripheral analgesic effect.

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