



Antinociceptive, anti-inflammatory and antidiarrheal activities of ethanolic calyx extract of *Hibiscus sabdariffa* Linn. (Malvaceae) in mice

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Objective: To evaluate the antinociceptive, anti-inflammatory and antidiarrheal activities of the ethanolic calyx extract of *Hibiscus sabdariffa* Linn. in mice.

Methods: In the present study, the dried calyces of *H. sabdariffa* were subjected to extraction with 95% ethanol and the extract was used to investigate the possible activities. Antinociceptive activity of the extract was evaluated by using the acetic acid-induced writhing test. The anti-inflammatory effect of the extract was tested by using the xylene-induced ear edema model mice. Castor oil-induced diarrheal model mice were used to evaluate the antidiarrheal activity of the extract.

Results: In acetic acid-induced writhing test, the extract produced inhibited writhing in mice significantly compared with the blank control ($P < 0.01$). The extract showed significant inhibition of ear edema formation in xylene-induced ear edema model mice in a dose-related manner compared with the blank control ($P < 0.01$). The extract demonstrated a significant antidiarrheal activity against castor oil-induced diarrheal in mice in which it decreased the frequency of defecation and increased the mean latent period at the doses of 250 and 500 mg/kg body weight ($P < 0.01$).

Conclusion: The above mentioned findings indicate that the calyx extract of *H. sabdariffa* possesses significant antinociceptive, anti-inflammatory and antidiarrheal activities that support its uses in traditional medicine.

Keywords: *Hibiscus*; plant extracts; analgesics; anti-inflammatory agents; antidiarrheals; mice

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Hibiscuss sabdariffa is a medicinal herb of Malvaceae family. It is available in all parts of the world and cultivated for its leaf, fleshy calyx, seed and fibre. The dried red calyx of this plant is commonly known as rosella in Australia, mesta in Indian subcontinent, chin baung in Myanmar, krajeab in Thailand, zobo in Nigeria, karkade in Egypt, Saudi Arabia and Sudan and sorrel in Latin America^[1]. It contains anthocyanins, β -carotene, ascorbic acid, protocatechuric acid, mucilage, gossypetin, hibiscine chlorideare, calcium citrate and cyanogenic glycoside^[1, 2]. *H. sabdariffa* has been traditionally used for high blood pressure, liver diseases, fever, ulcers, abscesses, anemia, etc^[3]. It has also been reported to be diuretic, astringent, antiseptic, aphrodisiac, cholagogue, demulcent, digestive, emollient, purgative, hypocholesterolemic, mucolytic, laxative, refrigerant, sedative, stomachic and tonic^[4, 5].

Previous scientific studies have reported that different parts of *H. sabdariffa* possess antioxidant and hypotensive^[6], anxiolytic and sedative^[7], antipyretic and antiatherosclerotic^[8], antihyperlipidemic, hepatoprotective, antibacterial, antioxidant, anticancer, anticlastogenic^[11], toxicity and immunomodulatory^[9], antihypertensive^[10], antimutagenic^[11], antispasmodic^[12], cytotoxicity and antibacterial^[13] effects. But scientific investigations on antinociceptive, anti-inflammatory and antidiarrheal activities of calyx extract of this plant have not yet been performed which may support its uses in traditional medicine. The present study was therefore undertaken to evaluate possible antinociceptive, anti-inflammatory and antidiarrheal activities of ethanolic calyx extract of *H. sabdariffa* in mice.

1 Materials and methods

1.1 Plant material collection and identification

The calyx of the plant was collected from Shyamnagar under the district of Satkhira of Bangladesh in December 2009 at day time. During the collection process, the calyxes were not washed or cleaned by water due to chance of hydrolysis, oxidation and other types of chemical degradation. The soils and dusts that were attached to the calyxes were removed by hand shaking. During the collection process, any type of adulteration was strongly prohibited. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh and tagged with the accession No. 33858. The voucher specimen is deposited in the Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna, Bangladesh.

1.2 Plant extract preparation Collected calyxes were separated from undesirable plant parts and materials. The calyxes were dried by air drying for 10 d. After drying, the calyxes were ground into coarse powder with the help of a grinder. The plant powder was stored in an airtight vessel

and kept in a cool and dry place. A glass jar with plastic cover was taken and washed thoroughly and 100 g of the dried powder was taken into the jar. 95% ethanol (500 mL) was poured into the jar up to 2.54 cm height above the sample surface as it can sufficiently cover the sample surface. The plastic cover with an aluminum foil was closed properly to resist the entrance of air into the jar and to avoid extraction of chemicals in contact with the solvent. This process was performed for 10 d. The jar was shaken several times during the process to get better extraction. After the extraction process the plant extract was filtered by a piece of clean and white cotton material twice. Then it was filtered through Whatman filter paper. The filtrate was collected in a beaker and evaporated under ceiling fan until dried. It rendered a gummy concentrate of deep red color. Finally, the extract was stored at 4 °C until use.

1.3 Experimental animals Young Swiss-albino mice aged 4 to 5 weeks, average body weight 20 to 25 g were used for the experiment. The mice were purchased from the animal house of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). They were kept in a standard environmental condition for one week in the animal house of the Biotechnology and Genetic Engineering Discipline, Khulna University, Bangladesh for adaptation. The animals were provided with standard laboratory conditions (relative humidity 55% to 60%, room temperature (25±2) °C and a 12 h light/dark cycle). All the experiments were conducted in an isolated and noiseless condition. This study was conducted according to the guidelines of Institutional Animal Ethics Committee^[14].

1.4 Chemicals and Drugs Glacial acetic acid and xylene were purchased from Sigma chemicals, USA. The standard drugs diclofenac sodium and loperamide were collected from Square Pharmaceuticals Ltd., Bangladesh.

1.5 Phytochemical screening Test of different chemical groups presenting in the extract represents the preliminary phytochemical studies. Preliminary phytochemical analysis of the ethanolic calyx extract of *H. sabdariffa* was carried out by using the standard procedure^[15, 16].

1.6 Antinociceptive activity The antinociceptive activity of the plant extract was evaluated by using the acetic acid-induced writhing test in mice following Ahmed *et al*^[17]. Mice were randomly selected and divided into 4 groups of 5 mice in each group. Mice of group I served as the blank control and received 1% Tween-80 orally at the dose of 10 mL/kg body weight; mice of group II served as the positive control and received diclofenac sodium orally at the dose of 25 mg/kg body weight; mice of group III and group IV as test groups received ethanolic calyx extract of *H. sabdariffa* at doses of 250 and 500 mg/kg body

weight, respectively. The rationale of choosing the dosage is according to crude drugs that had been traditionally used more or less at these doses in humans for curing various diseases. A 30 min interval was given to ensure proper absorption of the administered substances. Then the writhing-inducing chemical, acetic acid solution (0.7%, 10 mL/kg) was administered intraperitoneally to each mouse. An interval of 5 min was given for absorption of acetic acid and the number of writhing was counted for 15 min. The mice did not always perform full writhing. The incomplete writhing was taken as a half-writhing, so two half-writhings were taken as one full writhing. This is why total writhing was halved to convert all writhing to full writhing or real writhing.

1.7 Anti-inflammatory activity Xylene-induced ear edema model mice were used to assess the anti-inflammatory activity of the plant extract following the method described by Dev *et al*^[18]. Experimental mice were divided as the previous test: administered vehicle (1% Tween 80 in water, 10 mL/kg body weight); standard drug (diclofenac sodium, 10 mg/kg body weight) and two different doses of plant extract (250 and 500 mg/kg body weight). One hour after administration of the above drugs, 0.01 mL of xylene was injected to the anterior and posterior surfaces of the right ear of each mouse. One hour after xylene injection, mice were sacrificed and both treated and untreated ears were cut down by using a 7 mm diameter cork borer as circular sections and weighed. The weight difference between untreated and treated ear sections was calculated.

1.8 Antidiarrheal activity Castor oil-induced diarrheal model mice were used to evaluate possible antidiarrheal activity of the extract following the method described by Chatterjee^[19]. At first, all the mice were screened after oral administration of 0.5 mL of castor oil and only those showing diarrheal were selected and randomly divided into 4 groups of 5 mice in each group. Group I or the control received only distilled water containing 1% Tween-80 at a dose of 10 mg/kg body weight; group II or the positive control received standard antimotility drug loperamide at a dose of 50 mg/kg body weight as oral suspension. The two test groups were treated with suspension of calyx extract of *H. sabdariffa* at oral doses of 250 and 500 mg/kg body weight. The mice were fed with the samples 1 h prior to the oral administration of

castor oil. Mice of each group were placed in separate cages with adsorbent paper beneath and examined for the presence of diarrhoea every hour in the 4-hour study after the castor oil administration. The present investigation was done for 4 h for studying the delay of the onset of diarrhea episode or decrease of the frequency of defecation. Number of stools or any fluid material that stained the adsorbent paper were counted at each successive hour during the 4-hour period and were noted for each mouse. The latent period of each mouse was also counted.

1.9 Statistical analysis The data were presented as $\bar{x} \pm s_{\bar{x}}$. Results were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's *t* test for multiple comparisons. The significant difference was considered at $P < 0.05$.

2 Results

2.1 Phytochemical screening Preliminary phytochemical screening of the ethanolic calyx extract of *H. sabdariffa* revealed the presence of reducing sugars, tannins, flavonoids, glycosides, alkaloids, saponins, and steroids.

2.2 Antinociceptive activity In the acetic acid-induced writhing test, the ethanolic calyx extract of *H. sabdariffa* showed significant dose-dependent writhing inhibition compared with the control group. Maximum writhing inhibition was 66.85% at the dose of 500 mg/kg body weight, which is comparable to diclofenac sodium 78.45% at the dose of 25 mg/kg body weight (Table 1).

2.3 Anti-inflammatory activity In xylene-induced ear edema model mice, the ethanolic calyx extract of *H. sabdariffa* produced significant inhibition on ear edema formation in a dose-related manner compared with the blank control group. It caused 18.00% and 27.50% inhibition of ear edema formation at the doses of 250 and 500 mg/kg body weight, respectively (Table 2).

2.4 Antidiarrheal activity The effects of ethanolic calyx extract of *H. sabdariffa* on the latent period of castor oil-induced diarrheal model mice are presented in Table 3. The extract produced a significant increase in the latent period in comparison with the blank control at doses of 250 and 500 mg/kg body weight ($P < 0.01$). It also significantly reduced the total number of faeces as well as of diarrhoeic faeces in a dose-dependent manner compared with the blank control ($P < 0.01$) (Table 4).

Table 1 Effects of antinociceptive activity of ethanolic calyx extract of *Hibiscus sabdariffa* in mice

Group	<i>n</i>	Number of writhing ($\bar{x} \pm s_{\bar{x}}$)	Inhibition rate (%)
Blank control (AA (10 mL/kg, i.p.)+vehicle (10 mL/kg, p.o.))	5	36.20±2.03	00.00
Positive control (AA (10 mL/kg, i.p.)+diclofenac sodium (25 mg/kg, p.o.))	5	7.80±1.11**	78.45
Test 1 (AA (10 mL/kg, i.p.)+plant extract (250 mg/kg, p.o.))	5	18.00±1.45**	50.27
Test 2 (AA (10 mL/kg, i.p.)+plant extract (500 mg/kg, p.o.))	5	12.00±0.79**	66.85

** $P < 0.01$, vs blank control group. AA: acetic acid; i.p.: intraperitoneally; p.o.: per oral.

Table 2 Anti-inflammatory effect of ethanolic calyx extract of *Hibiscus sabdariffa* on xylene-induced ear edema

Group	n	Increased weight ($\bar{x} \pm s_{\bar{x}}$, mg)	Inhibition rate (%)
Blank control (xylene (0.01 mL, injection)+vehicle (10 mL/kg, p.o.))	5	10.00±0.32	00.00
Positive control (xylene (0.01 mL, injection)+diclofenac sodium (10 mg/kg, p.o.))	5	6.50±0.24**	35.00
Test 1 (xylene (0.01 mL, injection)+plant extract (250 mg/kg, p.o.))	5	8.20±0.37**	18.00
Test 2 (xylene (0.01 mL, injection)+plant extract (500 mg/kg, p.o.))	5	7.25±0.26**	27.50

** P<0.01, vs blank control group. p.o. : per oral.

Table 3 Effects of ethanolic calyx extract of *Hibiscus sabdariffa* on latent period of diarrheal induction in castor oil-induced diarrheal mice

Group	n	Mean latent period ($\bar{x} \pm s_{\bar{x}}$, h)	Increase in latent period (%)
Blank control (castor oil (0.5 mL, p.o.)+vehicle (10 mL/kg, p.o.))	5	0.72±0.03	00.00
Positive control (castor oil (0.5 mL, p.o.)+loperamide (50 mg/kg, p.o.))	5	1.66±0.08**	56.62
Test 1 (castor oil (0.5 mL, p.o.)+plant extract (250 mg/kg, p.o.))	5	0.98±0.05**	26.53
Test 2 (castor oil (0.5 mL, p.o.)+plant extract (500 mg/kg, p.o.))	5	1.22±0.04**	40.98

** P<0.01, vs blank control group. p.o. : per oral.

Table 4 Effects of ethanolic calyx extract of *Hibiscus sabdariffa* on frequency of defecation in castor oil-induced diarrheal mice

Group	n	Mean number of stools ($\bar{x} \pm s_{\bar{x}}$)	Inhibition rate (%)
Blank control (castor oil (0.5 mL, p.o.)+vehicle (10 mL/kg, p.o.))	5	17.80±1.19	00.00
Positive control (castor oil (0.5 mL, p.o.)+loperamide (50 mg/kg, p.o.))	5	5.21±0.89**	70.78
Test 1 (castor oil (0.5 mL, p.o.)+plant extract (250 mg/kg, p.o.))	5	12.20±1.08**	31.46
Test 2 (castor oil (0.5 mL, p.o.)+plant extract (500 mg/kg, p.o.))	5	9.60±1.22**	46.06

** P<0.01, vs blank control group. p.o. : per oral.

3 Discussion

In this study, the ethanolic calyx extract of *H. sabdariffa* was tested to investigate the possible pharmacological activities such as antinociceptive, anti-inflammatory and antidiarrheal activities. Antinociceptive activity was tested by using acetic acid-induced writhing test in mice. This test is used for detecting both central and peripheral analgesia^[18]. Intraperitoneal injection of acetic acid causes pain and localized inflammation through production of prostaglandins production, mainly prostacyclin (PGI₂) and prostaglandin-E (PG-E), which have been reported to stimulate the Aδ-fibres that cause a sensation of sharp well localized pain^[20, 21]. Acetic acid has also been reported to cause pain through activation of chemosensitive nociceptors or irritation of visceral surface that causes to liberate histamine, bradykinin and serotonin^[22]. Diclofenac sodium as a standard analgesic drug acts by inhibiting the synthesis of prostaglandin. Any agent that lowers the number of writhing will demonstrate analgesia by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition. The ethanolic calyx extract of *H. sabdariffa* significantly reduced the number of writhing in mice induced by the injection of acetic acid in a dose-dependent manner. Therefore, the result of the acetic acid-induced writhing model mice suggests that the extract may inhibit the writhing via inhibition of prostaglandin synthesis.

The extract was investigated to evaluate the

anti-inflammatory activity by using the xylene-induced ear edema mice. Xylene causes acute inflammation through the histopathological changes and results in increased thickness of the ear tissues in mice^[18]. In this study the ethanolic calyx extract of *H. sabdariffa* significantly inhibited ear edema formation in xylene-induced ear edema model mice. This inhibition can be considered a direct evidence supporting the anti-inflammatory efficacy of the ethanolic calyx extract of *H. sabdariffa* through reducing vasodilation and so that improving edematous condition.

Antidiarrheal activity of the ethanolic calyx extract of *H. sabdariffa* was also investigated by using the castor oil-induced diarrhea model mice and the extract showed potent antidiarrheal activity that supports its traditional use in the treatment of diarrhea. Castor oil is known to cause diarrhea due to its most active component ricinoleic acid. The liberation of ricinoleic acid from castor oil by lipase enzyme irritates the intestinal mucosa to cause inflammation and release of prostaglandin and nitric oxide that stimulate motility and secretion of electrolyte and water^[23, 24]. Several other mechanisms have been reported to cause diarrhea by castor oil including inhibition of intestinal Na⁺-K⁺-ATPase activity, activation of adenylate cyclase or mucosal cAMP-mediated active secretion, and platelet-activating factor^[25]. The present study showed that the ethanolic calyx extract of *H. sabdariffa* possesses significant anti-inflammatory properties. It is possible that the antidiarrheal action exerted by this extract may be related to

the inhibition of prostaglandin formation. However, confirmation through further studies is needed before such assertion is made.

Preliminary phytochemical screening of ethanolic calyx extract of *H. sabdariffa* showed the presence of reducing sugars, steroids, glycosides, alkaloids, saponins, flavonoids, and tannins. Alkaloids, flavonoids, saponins and tannins have been reported to have multiple pharmacological effects such as antinociceptive^[26-28], anti-inflammatory^[29, 30], antioxidant^[31] and antidiarrheal^[24, 32] activities. Therefore, antinociceptive, anti-inflammatory and antidiarrheal effects of the extract may be due to the presence of flavonoids, tannins and alkaloids either singly or in combination.

4 Conclusion

According to the above mentioned results, it can be concluded that the ethanolic calyx extract of *H. sabdariffa* possesses significant antinociceptive, anti-inflammatory and antidiarrheal activities that support this plant in traditional medicine use. This study also suggests further investigation to isolate most bioactive compounds responsible for the uses of this plant as traditional medicine.

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6 Competing interests

The authors declare that they have no competing interests.

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玫瑰茄花萼乙醇提取物的抗痛觉敏感、抗炎及止泻作用的实验研究

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目的: 研究玫瑰茄(*Hibiscus sabdariffa*)花萼的乙醇提取物的抗痛觉敏感、抗炎及止泻作用。

方法: 使用 95% 乙醇提取玫瑰茄花萼干品用于测定其功效。用小鼠扭体实验检测其抗痛觉敏感作用, 二甲苯致耳水肿模型小鼠检测其抗炎作用, 蓖麻油致腹泻模型小鼠检测其止泻作用。

结果: 在乙酸致小鼠扭体实验中, 玫瑰茄花萼的乙醇提取物对小鼠扭体的抑制与空白对照组相比差异有统计学意义($P < 0.01$), 对二甲苯致耳水肿模型小鼠的耳水肿的抑制与空白对照组相比差异有统计学意义($P < 0.01$), 且显著减少了蓖麻油致腹泻小鼠的排便次数并增加了排便间隔时间($P < 0.01$)。

结论: 本研究的结果证实了玫瑰茄花萼的乙醇提取物具有显著的抗痛觉敏感、抗炎及止泻作用, 验证了其在传统医学中的应用。

关键词: 木槿属; 植物提取物; 镇痛药; 抗炎剂; 止泻药; 小鼠