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**Original article**

# Analgesic and Anti-Inflammatory Activity of *Commelina benghalensis* Linn.

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*Commelina benghalensis* is a perennial herb, used in the traditional medicine system for the treatment of various ailments like leprosy, sore throat, opthalmia, burns, pain and inflammation and also used as depressant, demulcent, emollient and laxative. In the present study, ethanol extract of *C. benghalensis* roots was pharmacologically investigated to evaluate peripherally acting analgesic activity by acetic acid-induced writhing in Swiss Albino mice and centrally acting analgesic activity by hot-plate and tail-flick tests in mice. Anti-inflammatory activity was also evaluated using the carrageenan-induced mice paw edema model. In all the experiments, the extract was administered orally at the doses of 250 and 500 mg/kg body-weight. The ethanol roots extract demonstrated a significant ($P<0.0001$) inhibition of writhing as compared with the control group in acetic acid-induced writhing test in mice. The extract also significantly ($P<0.0001$) raised pain threshold level in both hot-plate and tail-flick tests in mice. Analgesic activity was in dose dependent manner in all the experimental models. The extract exhibited significant ($P<0.0001$) inhibition of paw edema at both doses after carrageenan administration, which revealed potential anti-inflammatory activity of the extract in dose dependent manner. The experimental results demonstrated that the ethanol extract possesses potential analgesic and anti-inflammatory activities.

**Keywords:** *Commelina benghalensis*, Commelinaceae, analgesic, Writhing, Hot-plate, Tail-flick, Anti-inflammatory.

## *Commelina benghalensis* Linn.'in Analjezik ve Anti-inflamatuvar Aktivitesi

*Commelina benghalensis* lepra, boğaz ağrısı, göz enflamasyonu, yanıklar, ağrı ve enflamasyon gibi çeşitli rahatsızlıkların tedavisinde ve yatıştırıcı, demulsan, emoliyan ve laksatif amaçlarla geleneksel tıpta kullanılan çok yıllık bir bitkidir. Bu çalışmada, bitkinin köklerinden etanol ile hazırlanmış ekstre farmakolojik olarak, Swiss Albino farelerde asetik asitle indüklenmiş ağrıya karşı analjezik aktivitede periferal etkisi ve hot-plate ve tail-flick testleri ile farelerdeki analjezik aktivitede santral etkisi açısından incelenmiştir. Antiinflamatuvar aktivitesi ise farelerde karragen ile indüklenmiş ayak ödemi modeli ile değerlendirilmiştir. Tüm deneylerde ekstre oral yolla 250 ve 500 mg/kg dozlarında uygulanmıştır. Ekstre asetik asit ile indüklenmiş modelde kontrol grubu ile kıyaslandığında önemli bir inhibisyon ($p<0.0001$) göstermiştir. Hot-plate ve tail-flick testlerinde ise ağrı eşiği seviyesini önemli oranda yükselttiği ($p<0.0001$) görülmüştür. Tüm deney modellerinde analjezik aktivitenin doz bağımlı olduğu belirlenmiştir. Ayrıca her iki dozda da karragen uygulandıktan sonra ayak ödeminde önemli oranda inhibisyon ($p<0.0001$) görülmüş, ekstrenin doza bağlı antiinflamatuvar aktiviteye sahip olduğu belirlenmiştir. Deneysel sonuçlar etanol ekstresinin güçlü analjezik ve antiinflamatuvar aktivitelere sahip olduğunu göstermiştir.

**Anahtar kelimeler:** *Commelina benghalensis*, Commelinaceae, Analjezik, Kıvranma, Hot-plate, Tail-flick, Antiinflamatuvar.

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## INTRODUCTION

*Commelina benghalensis* Linn. (Commelinaceae) is a perennial herb native to tropical Asia and Africa, commonly known as Benghal dayflower or Dew flower. It is large, straggling annual herb up to 40 cm long with rooting at basal nodes and characterized by attractive small bluish-violet flowers. Leaves are ovate-elliptic or oblong, shortly triangular, bright green in color and 4-7 cm long. The spathes are green, funnel-shaped, compressed and about 1.5 cm long. Capsules are broadly ovoid-oblong and 4-5 mm long. Seeds are ovoid.

*C. benghalensis* is used in traditional medicine system to treat various ailments. It is used for the treatment of headache, constipation, leprosy, fever, snake bite and jaundice (1-3). It is also used in the treatment of mouth thrush (4), insanity (5), epilepsy (6) and psychosis (7). In Lesotho it is applied to treat infertility in women and in India it is used as bitter, laxative, anti-inflammatory, demulcent, emollient and depressant (8). In China it is used as diuretic and febrifuge (9). In Pakistan it is used as vegetable (10). In Nepal paste of the plant is used to treat burns and juice of roots is used to treat indigestion (11).

In a previous study, antimicrobial activity of aqueous extract of *C. benghalensis* was evaluated (12). Potential sedative and anxiolytic activities of different fractions of the plant are reported in the literature (13). The plant is also reported with remarkable antioxidant, antitumor and anticancer activity (1, 14, 15). Protective activity of roots extract against paracetamol induced hepatic damage in Wistar rats has been reported (16). Phytochemical investigations on *C. benghalensis* have been revealed the presence of alkaloid, volatile oil, wax (17), vitamin C, vitamin A and $\beta$-carotene (18).

The present study was undertaken to investigate both peripheral and central analgesic and anti-inflammatory activities of the ethanol extract of *C. benghalensis* roots in different *in vivo* experimental models as well as to justify traditional medicinal uses.

## EXPERIMENTAL

*Plant material*

The roots of *C. benghalensis* were collected from Gallamari, Khulna in July 2010 and were authenticated by the experts at Forestry and Wood Technology Discipline (FWT), Khulna University, Khulna, Bangladesh, where a voucher specimen (Accession number- 37549) has been submitted for future reference and further study.

*Extraction*

After collection, all unwanted plant parts were removed and washed. Then the roots were sun dried for several days to remove moisture. After drying, the roots were cut into small pieces and powdered by Hammer mill. Then the powdered materials were socked in ethanol for 5 days with occasional shaking. After maceration, the plant debris was removed by filtration using cotton plug to get clear solution. Rotary vacuum evaporator was used to evaporate solvent at 50 °C to get crude extract. Then the crude extract has been stored in refrigerator at 4 °C until the pharmacological investigations were started. The roots yielded 4.56% extract of dried plant material.

*Experimental animals*

Young Swiss Albino mice of both sexes weighing 18-25 g were procured from International Center for Diarrheal Diseases and Research, Bangladesh (ICDDR, B), Dhaka, Bangladesh. The mice were acclimatized for 7 days under standard housing conditions (25±1 °C; 50-56% relative humidity with 12:12 h light/dark cycle) in animal house of Pharmacy Discipline, Khulna University. They were fed with standard ICDDR, B formulated rodent pellet food and had free access to tap water. The animals were habituated to standard laboratory conditions for 24 h prior to the pharmacological investigations to minimize any kind of unwanted and faulty response. Experiments on animals were carried out according to the guidelines of the Animal Ethics Committee, Life Science School, Khulna University, Khulna, Bangladesh (004/P/KU/2013).

*Chemicals and drugs*

Tween-80 was purchased from Loba Chemie Pvt Ltd, India. Carrageenan was purchased from Sigma-Aldrich, USA. Acetic acid was purchased from Merck, Germany. Diclofenac sodium and indomethacin were obtained from Beximco Pharmaceuticals Ltd, Bangladesh. Morphine was obtained from Popular Pharmaceuticals Ltd, Bangladesh.

*Evaluation of analgesic activity*

Analgesic activity was evaluated using three different established *in vivo* experimental models namely acetic acid-induced writhing, hot-plate and tail-flick tests. In these tests experimental animals were screened based on their susceptibility to these models and divided into four groups (control, reference and two test groups) consisting of six mice in each group. 1% Tween-80 in distilled water at the dose of 10 ml/kg body-weight was administered to the control group. Extract at the doses of 250 and 500 mg/kg body-weight were administered to the test groups. Tween-80 (1%) and extract were administered orally. In writhing test, diclofenac sodium (25 mg/kg body-weight, p.o.) was administered as standard to the reference group. In hot-plate and tail-flick tests, morphine (5 mg/kg body-weight, i.p.) was administered to the reference group.

*Writhing test*

After 30 min of the administration of the required treatments to the respective experimental groups, the mice were treated with 0.6% acetic acid at the dose of 10 mL/kg body-weight to each mouse at intraperitoneal route to produce abdominal contraction (19). After 5 min of acetic acid administration, the number of writhes was counted for each mouse for 10 min and percentage of inhibition of writhing was calculated to assess analgesic activity.

*Hot-plate test*

Mice having reaction time of 3-5 sec when placed in hot-plate maintained at 55±0.5 °C were selected for the present pharmacological assessment. All the required treatments were administered to the respective experimental groups. Reaction time or latency period for each mouse was recorded at 0, 30, 60, 90 and 120 min when mice licked their paws or jumped from the plate (20). To avoid accidental paw damage, a cut off period of 15 sec was considered. Analgesic activity was measured by comparing with the control group.

*Tail-flick test*

The present pharmacological assessment of analgesia was carried out according to the method described by Aydin et al. (1999) (21). Selected mice were divided into four groups and received respective treatments. In this test 3 cm of the tail of each mouse was immersed in water bath maintained at the temperature of 55±0.5 °C to record reaction time when mice withdrew their tail from warm water. The reaction time was recorded at 0, 30, 60, 90 and 120 min. Analgesic activity of the test groups were measured by comparing with the control group.

*Evaluation of anti-inflammatory activity*

Carrageenan-induced paw edema model was used to evaluate anti-inflammatory activity of the ethanol extract (22). Selected experimental mice were divided into four groups consisting of six mice in each group. Test groups received the ethanol extract at the doses of 250 and 500 mg/kg body-weight, reference group received indomethacin at the dose of 10 mg/kg body-weight and control group received 1% Tween-80 in distilled water at the dose of 10 mL/kg body-weight. All the treatments were administered orally. After 60 min of the administration of all the treatments, each mouse was injected with the suspension of carrageenan (0.5 mg/25 µL) in physiological saline into the subplanter tissue of the right hind paw of each mouse. The paw volume of each mouse was measured plethysmometrically (Ugo Basile, Italy) at 0 and 3 h. The difference between two measurements was considered as the volume of edema. The percentage of inhibition of paw edema was calculated using the following formula: % inhibition of paw edema = $(V_f-V_i/V_i) \times 100$, where $V_f$ is the volume of paw at 3 h (final volume) and $V_i$ is the volume of paw at 0 h (initial volume).

*Statistical analysis*

The statistical significance was assessed using Student's *t*-test. Results were expressed as mean ± SEM ($n$ = 6). Results were considered

as statistically significant when $P<0.05$ in comparison to control.

## RESULTS

*Evaluation of analgesic activity*

*Writhing test*

In acetic-acid induced writhing test, the ethanol extract showed significant ($P<0.0001$) inhibition of writhing in dose dependent manner. The extract exhibited 43.24 and 59.45% inhibition of writhing at the doses of 250 and 500 mg/kg body-weight, respectively as compared with the control group. The standard analgesic drug diclofenac sodium showed 72.97% inhibition of writhing at the dose of 25 mg/kg body-weight. Analgesic activity of the extract was highly comparable with diclofenac sodium (Table 1).

*Hot-plate test*

In hot-plate test, the ethanol extract significantly ($P<0.0001$) raised pain threshold up to 60 min at both doses of 250 and 500 mg/kg body-weight in comparison to control group. Standard drug morphine (5 mg/kg body-weight) also raised pain threshold significantly ($P<0.0001$) up to 90 min as compared with control. Analgesic activities of all the experimental groups are mentioned in Table 2.

**Table 1.** Effect of *C. benghalensis* roots on acetic acid induced writhing in mice.

| **Treatment** $n = 6$ | **Dose (mg/kg)** | **Number of writhing** | **% inhibition** |
|---|---|---|---|
| Control | --- | 18.5±0.76 | --- |
| Diclofenac sodium | 25 | 5.0±0.57* | 72.97 |
| Extract | 250 | 10.5±0.67* | 43.24 |
| Extract | 500 | 7.5±0.67* | 59.45 |

Values are expressed as mean ± SEM, SEM = Standard error of the mean, *: $P<0.0001$ as compared with control group (Student's *t*-test).

**Table 2.** Effect of *C. benghalensis* roots in hot-plate test in mice.

| **Treatment** $n = 6$ | **Dose (mg/kg)** | **Reaction time (sec)** | | | | |
|---|---|---|---|---|---|---|
| | | **0 min** | **30 min** | **60 min** | **90 min** | **120 min** |
| Control | --- | 4.33±0.07 | 4.53±0.04 | 4.45±0.08 | 4.40±0.07 | 4.44±0.06 |
| Morphine | 5 | 4.66±0.07* | 7.67±0.08** | 9.81±0.05** | 13.01±0.06** | 11.98±0.05** |
| Extract | 250 | 4.71±0.09* | 6.60±0.08** | 8.62±0.07** | 7.78±0.05** | 7.02±0.05** |
| Extract | 500 | 4.67±0.08* | 7.03±0.05** | 9.05±0.04** | 8.43±0.09** | 8.05±0.04** |

Values are expressed as mean ± SEM, SEM = Standard error of the mean, *: $P<0.01$, **: $P<0.0001$ as compared with control group (Student's *t*-test).

**Table 3.** Effect of *C. benghalensis* roots in tail-flick test in mice.

| Treatment $n = 6$ | Dose (mg/kg) | Reaction time (sec) | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min | 120 min |
| Control | --- | 3.31±0.04 | 3.39±0.03 | 3.50±0.06 | 3.41±0.05 | 3.55±0.05 |
| Morphine | 5 | 3.67±0.05* | 6.70±0.07** | 8.46±0.07** | 10.34±0.05** | 9.15±0.04** |
| Extract | 250 | 3.73±0.07* | 4.68±0.06** | 6.40±0.10** | 5.59±0.07** | 5.23±0.06** |
| Extract | 500 | 3.61±0.06* | 5.68±0.05** | 7.56±0.06** | 6.75±0.03** | 6.18±0.04** |

Values are expressed as mean ± SEM, SEM = Standard error of the mean, *: $P<0.001$, **: $P<0.0001$ as compared with control group (Student's *t*-test).

**Table 4.** Effect of *C. benghalensis* roots on carrageenan-induced paw edema in mice

| Treatment $n = 6$ | Dose (mg/kg) | Increase in paw volume (mean ± SEM) in µl | % inhibition of paw edema |
|---|---|---|---|
| Control | --- | 112.16±3.45 | --- |
| Indomethacin | 10 | 34.33±1.90* | 69.39 |
| Extract | 250 | 68.50±3.05* | 38.92 |
| Extract | 500 | 51.66±2.17* | 53.94 |

Values are expressed as mean ± SEM, SEM = Standard error of the mean, *: $P<0.0001$ as compared with control group (Student's *t*-test).

*Tail-flick test*

Analgesic activity results of the ethanol extract is shown in Table 3. The extract significantly ($P<0.0001$) raised pain threshold at both doses (250 and 500 mg/kg body-weight) up to 60 min, which was highly comparable with the standard drug morphine (5 mg/kg body-weight) that also significantly ($P<0.0001$) raised pain threshold up to 90 min of the observation period.

*Evaluation of anti-inflammatory activity*

The ethanol extract produced significant ($P<0.0001$) inhibition in carrageenan-induced paw edema in mice as compared with the control in dose dependent manner (Table 4). The extract showed 38.92 and 53.94% inhibition of paw edema at the doses of 250 and 500 mg/kg, respectively. Standard drug indomethacin also showed significant ($P<0.0001$) inhibition of paw edema in comparison to control. Indomethacin showed 69.39% inhibition of paw edema at the dose of 10 mg/kg body-weight.

## DISCUSSION

Pain is an unpleasant sensation often associated with every disease but in other sense it is the essential part of defense mechanism, because in favor of pain we can understand that something abnormal has been occurred in our physiological system including central and peripheral portion. Several drugs are available in the market to manage pain but most of them are associated with several unwanted side effects like NSAIDs cause gastric ulceration, steroidal drugs lead to hormonal imbalance and others like morphine and pathedine cause addiction and dependence. That is why researchers all over the world searching for more potent pain reliever from natural source and definitely without major side effects. *C. benghalensis* is being used in pain and inflammatory disorders in traditional medicine system as well as in Ayurveda without any major side effects for many years. In our present study, it was prime concern to justify traditional uses of the roots of this plant through pharmacological investigations.

Acetic acid-induced writhing test is very sensitive and most widely used *in vivo* model to evaluate peripheral analgesic activity at low dose levels than other methods (23, 24). Intraperitoneal injection of acetic acid increases the level of prostanoids, especially $PGE_2$ and $PGF_{2a}$ (25) as well as lipoxygenase

products in the peritoneal fluid (26). Release of these pain mediators is responsible for causation of abdominal contraction so called writhing in mice after acetic acid injection. The ethanol roots extract showed potential analgesic activity by reducing the number of writhes in dose dependent manner and the probable mechanism may be the inhibition of the release of pain mediators by acting on visceral receptors, sensitive to acetic acid like other commonly available NSAIDs. But this model is not sensitive enough to indicate the particular mechanism of analgesic activity because other agents like antihistamines (27) and myorelaxant (28) are able to reduce the pain induced by acetic acid.

Hot-plate and tail-flick tests are well established models to evaluate centrally acting analgesic activity. Neurogenic pain involves the stimulation of opoid receptors (μ, κ, γ) in spinal cord level (29). Drugs like morphine inhibit the stimulation of opoid receptors as well as block the transmission of neurotransmitters to manage neurogenic pain. The extract significantly increased pain threshold in both test models. The ability of the extract to reduce pain in these models suggests that it may possess potential centrally acting analgesic activity and the probable mechanism may be the blockage of opoid receptors in spinal cord level.

Edema is a pathophysiological inflammatory condition often associated with redness, swelling and pain as well as temperature elevation. The carrageenan-induced paw edema model is considered as a sensitive test protocol for anti-inflammatory agents, acting by the mediators of acute inflammation (30). Carrageenan-induced edema is a biphasic response involves the products of arachidonic acid metabolism as well as the production of reactive oxygen species (31). First phase is associated with the release of histamine, serotonin, and kinins in first hour whereas the second phase is attributed to the release of inducible cyclooxygenase, prostaglandins and lysosome enzymes in 2 to 3 hours (32). The extract potentially inhibited carrageenan-induced inflammation in mice in the 3rd hour in dose dependent manner which was strongly comparable to the standard drug indomethacin.

Analgesic and anti-inflammatory activities of the ethanol extract are also rationale with the reported chemical composition of *C. benghalensis*. A phytochemical research on *C. benghalensis* revealed the presence of carbohydrates, tannins, phlobatannins, glycosides, volatile oils, balsams, resins, flavonoids and saponins (33). The presence of resins, balsams and flavonoids demanded the possibility of analgesic and anti-inflammatory activities along with other activities (34, 35). There are several reports regarding the role of flavonoids and tannins isolated from the medicinal plants as promising analgesic and anti-inflammatory agents (36-38). So, the revealed activities of the extract may be attributed with the flavonoids and tannins. The present study provides scientific evaluation of these activities of the extract and rationales the anticipation of phytochemical research.

## CONCLUSION

The results of the present pharmacological investigations indicate that the ethanol extract of *C. benghalensis* roots possesses considerable analgesic and anti-inflammatory activities in all *in vivo* experimental models. It also provides a scientific rationale for the uses of *C. benghalensis* in traditional medicine system.

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