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Research Article

# Methanolic Extract of Peel of *Citrus maxima* Fruits Exhibit Analgesic, CNS Depressant and Anti-inflammatory Activities in Swiss Albino Mice

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**ABSTRACT**

**Objectives:** This study investigates analgesic, CNS depressant and anti-inflammatory activities of crude methanolic extract of Peel of *Citrus maxima* fruits. **Materials and Methods:** Methanolic extracts of *Citrus maxima* peel with different concentration were tested for analgesic activity in mouse model of acetic acid induced writhing and formalin induced licking and biting. Anti-inflammatory effect was tested by carrageenan induced paw edema model and the CNS depressant activity was evaluated by observing the reduction of locomotors activity by hole cross and open field test. **Results:** The extract, at 500 mg/kg, showed higher analgesic activity (73.34%) against acetic acid induced pain in mice while the standard reference drug Diclofenac sodium exhibited 87.13% activity at 10 mg/kg dose. The test dose at 500 mg/kg produced 48.23% protection in formalin induced hind paw licking and biting compared to 54.11% protection by the reference drug (10 mg/kg Diclofenac sodium). The anti-inflammatory effect of the extract was comparable to reference drug Ibuprofen and the effect was sustained for 2-4 hr. However, the extract also showed significant dose dependent CNS depressant activity. **Conclusion:** Methanolic extract of peel of Citrus maxima fruits has CNS depressant effect with moderate analgesic and anti-inflammatory properties.

**Key words:** Analgesic, Anti-inflammatory, CNS activity, *Citrus maxima*.

## INTRODUCTION

Several plants with medicinal propertiesareused to treat diseases.[1] Many rely on traditional medicines, chiefly plant-based, for there major health care needs.[2] The wide biological and medicinal properties, higher safety margins and affordability, make plant based medicines attractive for usein developing countries for primary health care.[3] *Citrus maxima* (pomelo, pomello, pummelo, pommelo, pamplemousse, batabi or jambura (Bengali), zambura (Sylheti), or shaddock) is an edible fruit that belongs to the family of Rutaceae. Its flesh is juicy, soft in texture and wealthy in nutrients and is endemic to tropical part of Asia.[4] Ancient texts mention its numerous uses and additionally describe its important role in Ayurvedic or natural medicine practices due to its vital constituents.[5] The fruit and pulp are cited as nontoxic, appetizer, and internal organ stimulant and abdomen tonic in ancient medical literature.[6] This plant has been used for the treatment of fatigue, diabetes, fever, insomnia, pharyngitis, carcinoma, coughs, and internal organ disorders in Philippines and surrounding geographic region. Recently leaves of this fruit are reported to have anticancer and CNS depressants activity[7,8] and its antioxidant potential is also used against paracetamol-evoked hepatotoxicity in rats.[9] Bactericidal activity of the leaves, peel and pulp is also reported.[10-13] While bark of this plant is reported to have anti-diabetic activity.[11] In this study we evaluated the analgesic, anti-inflammatory and central nervous system (CNS) depressant activity of methanolic extract of peel of *Citrus maxima* fruits.

## MATERIALS AND METHODS

### Chemicals

Diclofenac sodium, ibuprofen and diazepam were procured from Square Pharmaceuticals Company Ltd., Bangladesh. Acetic acid was procured from Merck, Germany. Normal saline water (0.9% NaCl), was purchased from Beximco Infusion Company Ltd., Bangladesh. BDH Chemicals Ltd., kindly provided tween-80, formalin, castor oil and carrageen. While all other chemicals were of analytical grade.

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## Animal

Swiss albino mice (male) weighing 25-30 g were obtained from the International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB) for this study. Mice were kept under ambient temperature with light (12 h) and followed by dark (12 h) cycle. The animals were acclimatized for one week prior to actual experiments. The study was approved by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh [Date: 4th September, 2014]. [Number of Approval: IAEC_UDA_33/2014]

## Plant material

The peel of *Citrus maxima* was collected during September 2014 from the area of Pabna, Bangladesh and was identified by a taxonomist of Bangladesh National Herbarium, Dhaka. Whose accession Number is 41681.

## Preparation of plant extract

After collection, peels of *Citrus maxima* fruits were thoroughly washed with water. Then the collected plant materials were chopped, dried, and powdered. About 500g of the powdered materials were soaked in 1.5 liter of methanol at room temperature for two weeks. Then the solution was filtered using filter cloth and What man's filter paper and concentrated with a rotary evaporator to yield brown granular extract. The brown granular extract is referred toas crude methanolic extract.

## Phytochemical evaluation

Freshly prepared methanolic extracts of peel of *Citrus maxima* fruits at small quantity were subjected to preliminary quantitative phytochemical investigation for the detection of phytochemicals such as alkaloids, glycosides, carbohydrates, proteins, phytosterols, tannins, flavonoids, saponins, phenols, gums and mucilages, fats and fixed oils using the standard methods reported previously.[13-16]

## Analgesic activity

### *Acetic acid-induced writhing method*

The mice were divided into four groups each containing five mice (n= 5). The analgesic activity of the samples was performed using acetic acid-induced writhing model in mice.[17] Test samples (300 and 500mg/kg body weight), vehicle (1% Tween 80 in water at the dose of 10 ml/kg p.o.) and Diclofenac sodium (10 mg/kg) were administered orally 30 min after the intra−peritoneal administration of 1% acetic acid. Then the mice were observed for specific contraction of the body referred to as 'writhing' for the next 20 min, complete 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, one full writhing was composed by two half-writhing. Diclofenac sodium (10 mg/kg) was used as a reference standard (positive control) and the number of writhes in each treated group was compared to that of a control group. The percent inhibition (% analgesic activity) was calculated by the equation {(A-B) /A} × 100

Where, A= Average number of writhing of the control group; B= Average number of writhing of the test group.

## Formalin induced licking and biting test

The anti-nociceptive activity of the drugs was determined by using the formalin test method.[18] The experimental animals (mice) were divided into four groups each containing five mice. 20 µl of 2.5% formalin was injected into the dorsal surface of the right hind paw 30 min before the administration of methanol extract of peel *Citrus maxima* fruits (300 and 500 mg/kg, p.o.), vehicle (1% Tween 80 in water at the dose of 10 ml/kg p.o.) and Diclofenac sodium (10 mg/kg, p.o.). After injection of formalin, the mice were observed for 30 min and the time spent licking of the injected hind paw was recorded. The first 5 min post formalin injection was mentioned as the early phase and the period between 15 and 30 min as late phase. The total time spent licking and biting of the injured paw (pain behavior) was measured with a stopwatch.

## Anti-inflammatory activity: Carrageenan-induced paw edema method

The mice were divided into four groups each containing five mice. 0.1 ml of 1% carrageenan was injected into the plantar surface of the right hind paw to induce acute inflammation.[18] The extract (300 and 500 mg/kg), normal saline (1 ml/kg) and Ibuprofen (10 mg/kg, i.p.) as the referral agents were administered 30 min after carrageenan injection. Vernier caliper was used to determine the diameter of edema by measuring the paw volume at 0, 1, 2, 3, and 4 h during the study season. The difference between the readings at time 1 h and different time interval was taken as the thickness of edema.

## CNS depressant activity: Hole cross test

The test was carried out by following the method described by Takagi *et al*.[19] A steel partition was fixed at the middle of a cage having a size of 30×20×14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the center of the cage. Twenty mice were divided into four groups with five mice in each group. Mice of group-I received vehicle (1% Tween-80in water at the dose of 10 ml/kg p.o.), group-II received diazepam at 1 mg/kg body weight (p.o.) while group-III and group-IV were treated with 300 and 500 mg/kg body weight (p.o.) of the extract. The number of mice passing through the hole from one chamber to another was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of test samples.

## CNS depressant activity: Open field test

This test was performed by following the method described by Gupta *et al*.[20] The animals were divided into control, standard and test groups (n = 5 per group). The control group received vehicle (1% Tween 80 in water at the dose of 10 ml/kg p.o.). The test group received the methanolic extract (at the doses of 300 and 500 mg/kg p.o.) and standard group received diazepam at the dose of 1mg/kg body weight orally. The animals were placed on the floor of an open field (100 cm×100 cm×40 cm h) divided into a series of squares. The number of squares visited by each animal was counted for 3 min at 0, 30, 60, 90 and 120 min during the study period.

## Statistical analysis

All the above assays were conducted in triplicate and repeated three times for consistency of results and statistical purpose. The data were expressed as Mean ± SD and analyzed by one-way analysis of variance (ANOVA) followed by Dennett's' test using SPSS software of 10 version. $P<0.05$ was considered statistically significant.

# RESULTS

## Phytochemical analysis

Phytochemical screening of methanolic extracts of peel of *Citrus maxima* fruits demonstrated the presence of carbohydrate(s), glycoside(s), phenol, tannin, protein(s), gum and mucilages (Table 1).

## Analgesic activity

### *Acetic acid induced writhing in mice*

The effect of methanol extract of peel of *Citrus maxima* fruits investigated against acetic acid induced writhing in mice is represented in Table 2. About 87.13% inhibition of writhing was observed in mice treated with the reference drug; Diclofenac sodium (10 mg/kg). The methanol extract of peel of *C. maxima* fruits significantly reduced the acetic acid induced abdominal constrictions and stretching in a dose dependent manner (group-III, IV) compared to that of control (group-I). The analgesic effect of the extract at a dose of 300mg/kg was comparable to that of a dose 10 mg/kg of Diclofenac sodium.

## Formalin induced hind paw licking in mice

The crude extract at 300 and 500 mg/kg body weight showed a significant dose-response reduction in the hind paw licking (Table 3) compared to that of control. The extract at both tested doses showed better activity as compared to reference standard Diclofenac sodium at 10 mg/kg dose.

## Anti-inflammatory activity: Carrageenan induced paw edema in mice

The extract exerted anti-inflammatory effect at the test dose of 300 and 500 mg/kg body weight which was comparable to that of the positive control group (Group-II) (Table 4). The percent inhibition of carrageenan-induced inflammation at that doses were relatively low for initial 1 h period but had more pronounced effect subsequently at 2-3 hr and was comparable to that of standard drug Ibuprofen at 10 mg/kg dose. Moreover, it is notable that the dose independent effect of the extract or slightly better anti-inflammatory effect at the dose of 300 mg/kg was found after 3-4 h of extract administration.

## CNS depressant activity: Hole-cross test

The CNS depressant activity of the extract measured by Hole-cross test is shown in Table 5. The CNS depressant effect of the extract was instantaneous compared to the reference drug diazepam, since the number of movements at 0 min was statistically significant but the number of movement of reference drug was not. Both doses (300 and 500 mg/kg) of extract showed significant CNS depressant effect for the time of experiment tested and followed a dose dependent response. The obtained result revealed that the methanol extract of peel of *C. maxima* fruits was potent CNS depressant under our experimental conditions.

## CNS depressant activity: Open-field test

The extract showing CNS depressant activity measured by the open-field test is shown in Table-6. The extract exhibited a decrease in the movements of the test animals at all dose levels. The results were statistically significant for all doses at 120 min and followed a dose-dependent response.

**Table 1: Preliminary phytochemical screening of the methanolic extracts of peel of *Citrus maxima* fruits.**

| Phytochemicals | Methanolic Extract |
|---|---|
| Alkaloids | - |
| Carbohydrates | + |
| Deoxysugar Cardiac glycosides | + |
| Anthracene glycoside | - |
| Saponins | + |
| Phytosterols | + |
| Phenols | + |
| Tannins | + |
| Flavonoids | + |
| Proteins and amino acids | + |
| Terpenes | + |

Here, "+" stands for Presence of Phytochemicals and "-"stands for Absent of Phytochemicals.

**Table 2: Effects of the methanol extract of peel of *Citrus maxima* fruits on acetic acid induced writhing in mice.**

| Groups | Dose mg/kg | No. of writhing | % inhibition |
|---|---|---|---|
| Group I | Vehicle | 54.4±1.07 | - |
| Group II | 10 (standard) | 7.2±1.25*** | 87.13 |
| Group III | 300 | 21.5±1.08** | 60.47 |
| Group IV | 500 | 14.5±1.11** | 73.34 |

Values are mean ± SEM; SEM=standard error of mean (n=5), Dunnet test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received Diclofenac Na (10 mg/kg body weight), Group III and Group IV were treated with 300 and 500 mg/kg methanolic extract (p.o) of peel of *Citrus maxima* fruits respectively. Here, *=significant, **=more significant, ***=most significant.

**Table 3: Effects of the methanolic extract of peel of *Citrus maxima* fruits on hind paw licking in the formalin test in mice.**

| Groups | Dose(mg/kg) | Early phase | % protection | Late phase | % protection |
|---|---|---|---|---|---|
| **Group I** | Vehicle | 28.8± 1.54 | - | 14.20±1.28 | |
| **Group II** | 10 (standard) | 12.2±1.14 | 66.66 | 7.60± 1.29** | 54.11 |
| **Group III** | 300 | 15± 1.89 | 47.91 | 11.80±1.14* | 30.58 |
| **Group IV** | 500 | 14.0 ± 1.10 | 51.38 | 8.80 ± 1.16** | 48.23 |

Values are mean ± SEM; SEM=standard error of mean (n = 5), Dunnet test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received Diclofenac Na (10mg/kg body weight), Group III, Group IV were treated with 300 and 500 mg/kg (p.o) methanol extract of peel of *Citrus maxima* fruits respectively. Here, *=significant, **=more significant.

**Table 4: Effect of methanol extract of peel of *Citrus maxima* fruits on carrageenan induced paw edema in mice.**

| Groups | | Oedema diameter(mm) | | | | | Inhibition (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | Dose (mg/kg) | 0 min | 1hr | 2hr | 3hr | 4hr | 0 min | 1hr | 2hr | 3hr | 4hr |
| Group I | Vehicle | 4.32±0.38 | 4.25± 0.51 | 4.05± 0.32 | 3.90± 0.40 | 3.80± 0.30 | | - | - | | |
| Group II | 10 | 2.42± 0.47 | 1.86± 0.46 | 1.44± 0.39 | 1.07±0.32** | 0.90± 0.29*** | 43.98 | 56.23 | 64.44 | 72.56 | 76.31 |
| Group III | 300 | 3.25±0.35 | 3.08±0.31 | 2.89±0.35 | 2.69±0.35** | 2.49±0.31** | 24.76 | 27.52 | 28.64 | 31.02 | 34.47 |
| Group IV | 500 | 3.11±0.30 | 2.99±0.42 | 2.77±0.44* | 2.54±0.35** | 2.33±0.30** | 28.43 | 29.64 | 31.60 | 34.87 | 38.68 |

Values are mean ± SEM; SEM=standard error of mean (n = 5) Dunnet test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received Ibuprofen 10 mg/kg body weight, Group III and Group IV group were treated with 300 and 500 mg/kg (p.o) methanolic extract of peel of *Citrus maxima* fruits respectively. Here, *=significant, **=more significant, ***=most significant.

**Table 5: Effect of methanolic extract of peel of *Citrus maxima* fruits on the whole cross test in mice.**

| Group | Dose (mg/kg) | Number of movements | | | | |
|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 90 min | 120 min |
| **Group I** | Vehicle | 10.40±1.44 | 8.41±1.06 | 8.11±1.25 | 7.09±1.34 | 6.60±1.06 |
| **Group II** | 1 | 8.30±1.06 | 6.40±1.06 | 4.80±0.91* | 3.97±1.47** | 3.11±1.25*** |
| **Group III** | 300 | 9.79±0.91 | 8.04±1.38 | 6.60±1.44 | 5.96±1.06* | 5.08±1.12** |
| **Group IV** | 500 | 9.01±1.38 | 7.79±0.74 | 6.02±0.94* | 4.97±1.04** | 4.09±0.91** |

Values are mean ± SEM; SEM=Standard error of mean. (n = 5); Dunnet test as compared to vehicle control. Group I animals received vehicle (1% Tween 80 in water), Group II received diazepam (1 mg/kg body weight), Group III and Group IV were treated with 300 and 500 mg/kg methanol extract (p.o) of peel of *Citrus maxima* fruits respectively. Here, *=significant, **=more significant, ***=most significant.

## DISCUSSION

The literature survey unconcealed that peel of *C. Maxima* fruits is extremely thought to be a universal remedy within the flavoring drugs with various spectrum of pharmacologic activity. Several mechanisms are involved in the regulation of pain.[21,22] The two completely different doses (300 and 500 mg/kg body weight) of methanolic extract of peel of *Citrus maxima* fruits showed significant analgesic (60.47% and 73.34%) activity. We envisage the extract exerted its analgesic effects by modulating both central and peripheral pain pathways.[23-25] Neuropathic pain was impacted by direct stimulation of nerve fibers that is the1$^{st}$part of biphasic formalin-induced nociception while the second part involves inflammatory pain mediated by autocoid, serotonin, histamine, bradykinin, cytokines (IL-1β, IL-6, TNF-α), eicosanoids and nitric oxide.[26] Crude extract of peel of *C. maxima* fruits showed inhibition at the primary part of atrogenic nociception in mice and this inhibition was superior to nonsteroidal anti-inflammatory drug. Therefore, we assume that the extract exhibited the analgesic impact by reducing hyper nociception iatrogenic pathway possibly by influencing several mediators of pain. The extract at the dose of 300 and 500 mg/kg had better protection (30.58% and 48.23% respectively) against licking and biting iatrogenic mice than standard nonsteroidal anti-inflammatory drug (54.11%). Carrageenan-induced paw edema is a model of acute inflammation and has biphasic response. The inflammation in this model is mediated by amino alkane, serotonin, and increased synthesis of autocoids within the broken tissue surroundings and the late part is sustained by prostaglandins, bradykinin, and leukotrienes.[26] During this study the methanolic extract of peel of *C. maxima* fruits at the dose of three hundred and five hundred mg/kg exhibited and sustained inhibition (34.47% and 38.68%) of paw edema at the 4$^{th}$ hr whereas the standard ibuprofen reported only 76.31% inhibition. The attainable mechanism of the ascertained medicament activity can be its ability to slightly cut back the discharge of amino alkane, 5-hydroxytryptamine or plant hormone like substances or synthesis of prostaglandins that is in line with the analgesic activity of the respective plant extract.

Increase in locomotors activity is attributed to rise in alertness. The study of locomotors activity, as measured by whole cross and open field tests, showed that the extract at the dose of three hundred and five hundred mg/kg attenuated the frequency and therefore the amplitude of movements of mice. Since, locomotors activity reflects excitability of the system,[27] the decrease in spontaneous motor activity may well be attributed to the sedative impact of the plant extracts.[28] The locomotors activity lowering impact was evident within the first observation (0 min) and continuing up to fourth observation amount (90 min). Most depression of locomotors activity was ascertained from the fourth (90 min) to fifth (120 min) observation point. Gamma-amino-butyric acid (GABA) is that the major repressive neurochemical within the central nervous system.[27] Several studies have reported the biological activity of plant containing flavonoids, saponins and tannins.[27]These phyto-constituents can mimic neuro-active steroids and can be ligands for the neurotransmitter receptors within the central system resulting in anxiolytic activity. It may be likely those neuro-active steroids like phyto-constituents may mediate the central nervous system depressant activity of methanolic extracts of peel of *C. maxima* fruits.

## CONCLUSION

Crude methanol extract of peel of *C. maxima* fruits possesses moderate analgesic, and CNS depressant activities. This work is a preliminary effort that needs further investigation together with characterization of active compounds and requires studies to optimize development of methanolic extract of peel of *C. maxima* fruits based analgesic and anti-inflammatory therapeutics.

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## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## CONTRIBUTION STATEMENT

M.I., M.N.A., J.A. R. and M.S.M. were directly related to conducting this research work. M.S.H., F.S., M.M.I., and M.M.H were also contributed during data generation, manuscript preparation and collection of the plant part. All the authors consent to the publication of this research work.

## ABBREVIATIONS USED

**CNS:** Central Nervous System; **ICDDRB:** International Centre for Diarrheal Disease Research, Bangladesh, **ANOVA:** Analysis of variance, **GABA:** Gamma-amino-butyric acid.

## SUMMARY

This study was undertaken to investigates analgesic, CNS depressant and anti-inflammatory activities of crude methanolic extract of Peel of *Citrus maxima* fruits. Methanolic extracts of *Citrus maxima* peel with different concentration were tested for analgesic activity in mouse model of acetic acid induced writhing and formalin induced licking and biting. Anti-inflammatory effect was tested by carrageenan induced paw edema model and the CNS depressant activity was evaluated by observing the reduction of locomotors activity by hole cross and open field test. Methanolic extract of peel of Citrus maxima fruits has CNS depressant effect with moderate analgesic and anti-inflammatory properties.

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