Bioactivities of stem bark of *Albizia chinensis* Osbeck. Merr., Chakua Koroi of Bangladesh

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*Albizia chinensis* Osbeck. Merr. (Family: Fabaceae) is quite abundant in Bangladesh. The plant has a history of its traditional use in scorpion and snake bite. This investigation was designed to analyze the analgesic, anti-diarrheal and anti-inflammatory potentials along with sleep inducing property of the crude methanol extract of stem bark of *Albizia chinensis* Osbeck. Merr. Peripheral analgesic activity was evaluated by inducing pain through intraperitoneal administration of acetic acid in writhing test. Tail-flick test protocol was followed to determine central analgesic activity. The tail flicking response to thermal stimulus was measured in this assay. *A. chinensis* extract was also tested for anti-diarrheal activity by inducing castor oil-induced diarrhea in Swiss albino mice model. Phenobarbitone-sodium induced sleeping time was determined to assess CNS depressant activity. The crude methanol extract (400 mg/kg body weight) demonstrated 85.71% inhibition of writhing as compared to 74.49% by diclofenac sodium. The crude extract (400 mg/kg dose) reduced diarrheal feces by 69.05% and revealed 53.03% elongation of the reaction time after 30 min of administration in tail flick test. At the same dose, the crude methanol extract exhibited 91.49% inhibition of rat paw edema at the 4th h of carrageenan injection. The phytocomponents responsible for observed activity should be isolated and involved in further assessment.

**Key words:** *Albizia chinensis*, analgesic, writhing, anti-diarrheal, anti-inflammatory, carrageenan, edema.

**INTRODUCTION**

*Albizia chinensis* Osbeck. Merr. (Synonyms: *A. stipulata* DC., *A. marginata* Lam. Merr.) is locally known as Chakua koroi. It is a flowering plant belonging to Fabaceae family. The plant is native to Southeastern Asia and invasive in Hawaii and Samoa. Traditionally, its leaf paste is used to treat scorpion and snake bite (Verma and Chauhan, 2007). According to Medicinal Plants Database of Bangladesh, the aerial parts of the plant possess spasmogenic and diuretic activities. The plant extract was reported to demonstrate significant...
antioxidant, antimicrobial, cytotoxic and anti-proliferative properties (Manosroi et al., 2012; Sharmin et al., 2014). Previous phytochemical studies with A. chinensis revealed the occurrences of quercetin 3’-O-β-D-glucopyranosyl-3-O-rutinoside, kaempferol 3,7-di-O-beta-D-glucopyranoside, rutin, D-pinitol, luteolin 7- O-β-D-glucopyranoside, (+)-lyoniresinol 3α-O-β-D-glucopyranoside, (-)-lyoniresinol 3α-O-β-D-glucopyranoside, syringing, five triterpene saponins (albizzosides A–E), kaempferol-3-O-α-L-rhamnopyranoside, quercetin-3-O-α-L-rhamnopyranoside, luteolin, dimethoxyluteolin, lupeol, α-amyrin, stigmasterol, β-sitosterol, kaempferol and quercetin (Sharmin et al., 2014; Liu et al., 2009, 2010; Ghaly et al., 2010; Kolila et al., 2013). Some of these phytoconstituents have been reported to possess analgesic, anti-inflammatory and anti-diarrheal properties (Carvalho and Carvalho, 2001; Palanichamy and Nagarajan, 1990; Geetha and Varalakshmi, 2001; Knipping et al., 2012).

Bangladesh is blessed with plants with numerous bioactive compounds found to be useful for treating many diseases. We are trying to investigate the medicinal plants of Bangladesh on the basis of their long history of traditional uses (Sharmin et al., 2014, 2017, 2018). From this attempt, the present study was undertaken to evaluate A. chinensis stem bark extract for analgesic, anti-diarrheal, anti-inflammatory and sleep-inducing property for the first time. We, herein, report the results of our investigation.

**MATERIALS AND METHODS**

**Plant materials**

**Collection of plant materials**

A. chinensis stem bark was collected from Dhaka. This collection was identified in Salar Khan Herbarium, Department of Botany, University of Dhaka and a voucher specimen (DUSH–5386) has been preserved here for this collection.

**Extraction and fractionation**

At first, the stem bark was thoroughly cleaned. Then it was cut into small pieces. The pieces were sun-dried and ground into powder. 2.5 L methanol was used for maceration of the powder. The plant sample was kept in this condition for 7 days at room temperature. After 7 days, the sample was filtered and concentrated to yield the crude methanol extract.

**Animals**

Healthy Swiss-albino mice (average weight 25 g) were used to evaluate the analgesic, anti-diarrheal and sleep-inducing properties while Wistar albino rats (average weight 125 g) were used in anti-inflammatory activity assay. Healthy animals were collected from the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B). They were held in cages bedded with flake wood shavings. Standard laboratory environment (room temperature 25 ± 2°C; relative humidity 55-60%; 12 h light-dark cycle) was maintained. Animals had easy access to food and water. The animals were acclimatized to the laboratory environment for 15 days before involving them in any experiments. The animals were kept fasting overnight before they were picked up randomly for experiments (Hawk et al., 1954). In order to minimize physical and mental sufferings after completion of each experiment, the animals were euthanized using carbon dioxide gas and cervical dislocation method. Swiss Academy of Medical Sciences formulated Ethical Principles and Guidelines for Scientific Experiments on Animals (1995) were followed. All the experiments were performed under the approval of the Ethics Committee of State University of Bangladesh.

**Drugs and chemicals**

Morphine was procured from Gonoshastho Pharmaceuticals Ltd., Dhaka, Bangladesh. Diclofenac sodium BP, indomethacin BP, loperamide BP and pure phenobarbitone sodium were obtained from Incepta Pharmaceuticals Ltd., Bangladesh. The rest of the drugs, reagents and solvents were obtained from Sigma-Aldrich, Munich, Germany.

**Evaluation of peripheral analgesic potential by acetic acid induced writhing method**

The assay was conducted according to the method followed by Kaushik et al. (2012). Twenty healthy mice were divided among control and test groups. Mice that received 1% Tween-80 in saline mixture (10 ml/kg) constituted the negative control group while those that received diclofenac sodium (50 mg/kg body weight) formed the positive control group. The test groups received the crude methanol extract (200 and 400 mg/kg body weight doses). After 40 min, all mice were given 1% volume/volume acetic acid in distilled water (10 ml/kg dose) intraperitoneally. The animals were allowed to rest for the next 10 min. After that, the number of writhing exhibited by each animal was monitored for 10 min. The percent inhibition of pain induced by acetic acid was calculated according to the following formula:

\[
\text{Percentage inhibition} = \frac{[\text{Mean number of writhing (control)} - \text{Mean number of writhing (test)}] \times 100}{\text{Mean number of writhing (control)}}
\]

**Evaluation of central analgesic activity by tail flicking method**

In central analgesic activity assay, the last 1-2 cm of the tail of mice was immersed on the radiant heat source to observe the tail flicking response (Pizziketti et al., 1985). An end point of 15 s was set to prevent damage to the tail. Here, mice of negative control group received 1% Tween-80 in saline mixture (10 ml/kg body weight) while mice of positive control group received morphine (2 mg/kg body weight) by subcutaneous administration. Mice of treatment groups received the crude methanol extract (200 and 400 mg/kg body weight). After that, the tail withdrawal time was recorded at 30th, 60th and 90th min. The pain inhibition percentage (PIP) was calculated according to the following formula:

\[
\text{Pain inhibition percentage (PIP)} = \frac{(T_1 - T_2)}{T_2} \times 100\%
\]

where, \(T_1\) is post-drug latency and \(T_0\) is pre-drug latency.

**Anti-diarrheal activity by castor oil challenge**

The anti-diarrheal activity was evaluated by using castor oil to
induce diarrhea in mice (Shoba and Thomas, 2001). Here, twenty mice were divided into four groups. The negative control group received 1% Tween-80 in saline mixture (10 ml/kg); the group serving as positive control received loperamide (50 mg/kg) orally. Mice of treatment groups received the methanolic crude extract (200 mg/kg and 400 mg/kg body weight). According to this method, each mouse was fed with 1 ml of pure analytical grade castor oil to induce diarrhea. Each animal was placed in individual cage and the floor lining was changed at every hour to determine the number of fecal discharges of individual mouse for next four hours. The observed data were compared against that of control groups to evaluate the anti-diarrheal activity.

**Evaluation of anti-inflammatory activity**

The assay was conducted using carrageenan-induced edema model (Amdekar et al., 2012). Acute inflammation was induced using carrageenan. Twenty Wistar rats were divided into four groups. Before administering anything, the paw circumference of all the animals was measured by cotton thread method in which a cotton thread was looped round the paw of the rats and slightly tightened so that it was neither too loose nor too tight. Rats of negative control group were given saline at 100 ml/kg dose while those of positive control group received indomethacin (10 mg/kg) orally. Rats of treatment groups were treated with the methanolic crude extract (200 mg/kg and 400 mg/kg body weight doses). Later, all the rats were injected with 0.1 ml of 1% freshly prepared suspension of carrageenan into the plantar surface of the right hind paw to induce inflammation.

Paw circumference was measured at 1st, 2nd, 3rd and 4th hour after carrageenan injection. The length of the thread around the paw was then measured on a ruler and rounded off to the nearest centimeter. Mean increase in paw circumference was then noted for the respective time intervals. Paw circumference in control [\(C_0\) control] and sample groups [\(C_{t-C_0}\) treated] was determined. The percentage inhibition of paw edema was calculated by using the following formula:

\[
\text{% paw edema inhibition} = \left(\frac{\left(C_{t-C_0}\right)_{\text{control}} - \left(C_{t-C_0}\right)_{\text{treated}}}{\left(C_{t-C_0}\right)_{\text{control}}}\right) \times 100
\]

where, \(C_0\) paw circumference at zero time (before carrageenan injection), \(C_t\) = paw circumference at time \(t\), \(C_{t-C_0}\) = paw edema.

**Phenobarbitone- sodium induced sleeping time test**

The assay was conducted according to the method of Williamson et al. (1996). Mice of control group received 1% Tween-80 in saline mixture (10 ml/kg body weight) while the experimental groups were treated with the crude methanol extract (200 and 400 mg/kg body weight). After 30 min, all the mice were given phenobarbitone sodium (25 mg/kg body weight) intraperitonially to induce sleep. The time of onset of sleep and total sleeping time were recorded. The animals were observed for the time of onset of sleep (time between phenobarbitone administration to loss of righting reflex) and the duration of sleep (the time between the loss and recovery of righting reflex).

**Statistical analysis**

Three replicates of each sample were used for statistical analysis and all of the values are expressed as the mean± standard deviation (SD). The results were evaluated by a two-tailed non-parametric pair t-test.

**RESULTS**

This research was an effort to evaluate the crude methanol extract of *A. chinensis* stem bark for different bioactivities in animal model. The crude methanol extract of *A. chinensis* stem bark revealed statistically significant analgesic activity in animal model. The mean number of writhing was significantly lower \(p < 0.01\) in mice receiving the test samples when compared to that of negative control group. The crude methanol extract demonstrated 72.45 and 85.71% inhibition of writhing at 200 and 400 mg/kg body weight doses, respectively in contrast to the standard, diclofenac sodium inhibiting writhing by 74.49% (Table 1).

The crude methanol extract (400 mg/kg body weight) of *A. chinensis* demonstrated 53.03% elongation of the reaction time after 30 min of administration (Tables 2 and 3). The crude methanol extract of *A. chinensis* also showed anti-diarrheal property in castor oil-induced diarrhea in mice. The extract (400 mg/kg body weight) reduced diarrheal feces by 69.05 % which was comparable to the standard loperamide (77.20%) (Table 4). At a dose of 400 mg/kg body weight, the crude methanol extract exhibited extremely significant anti-inflammatory activity in carrageenan-induced rat paw edema model (91.49 % inhibition) which was comparable to the effect demonstrated by standard indomethacin revealing 93.62 % inhibition of paw edema (Table 5). *A. chinensis* extract decreased the total duration of phenobarbitone sodium–induced sleep in a dose dependent manner (Table 6).

**DISCUSSION**

The highly significant analgesic activity of *A. chinensis*...
could be attributed to a few of the compounds already reported from the species. Luteolin revealed analgesic activity after intrathecal and intracerebroventricular administration in neuropathic pain model (Hara et al., 2014) and in acetic acid-induced writhing assay on mice (Carvalho and Carvalho, 2001). It exhibited stronger anti-nociceptive properties than acetyl salicylic acid, acetaminophen, dipyrone and indomethacin (Block et al., 1998). Again, Cassia siamea Lam extract revealed noteworthy analgesic effect in hot plate assay, in which the flavonoid, luteolin had contributed to the extract’s analgesic potential (Nsonde et al., 2010). On the other hand, kaempferol 3-O-sophoroside revealed analgesic activity in tail clip, tail flick, tail immersion and acetic acid-induced writhing methods (Palanichamy and Nagarajan, 1990). Both luteolin and kaempferol have already been isolated from the species under investigation (Ghaly et al., 2010; Ghaly et al., 2010; Kokila et al., 2013).

Lupeol and lupeol linoleate demonstrated anti-inflammatory potential by showing a reduction in paw swelling in adjuvant arthritis and the outcome was comparable to indomethacin (Geetha and Varalakshmi, 2001). Lupeol and lupeol acetate have been reported to exhibit anti-nociceptive and anti-inflammatory activities in vivo and in vitro (Adzu et al., 2015; Chen et al., 2012). On the other hand, quercetin and isoquercitrin were found to be effective in allergies by acting as eosinophilic inflammation suppressors (Rogerio et al., 2007). Luteolin reduces production of IL-6 and other inflammatory mediators (Jang et al., 2008; Ando et al., 2009). It has been found to be effective in neuroinflammation (Jang et al., 2008). Moreover, kaempferol and kaempferol rhamnosides demonstrated anti-inflammatory potential (Rho et al., 2011). Lupeol, quercetin and isoquercitrin have already been isolated from the species under investigation (Sharmin et al., 2014; Ghaly et al., 2010; Kokila et al., 2013).

Luteolin was found effective in rotaviral infection which leads to severe diarrhea (Knipping et al., 2012). It has been also observed that luteolin has been isolated from many plant species that showed anti-diarrheal effect (Sheng et al., 2016; Sadraei et al., 2016, 2018; Mehmood et al., 2015; Khan et al., 2011; Meitei et al., 2009). Therefore, it can be assumed that luteolin might have contributed to the anti-diarrheal efficacy of those plant extract as well as to the plant under investigation.

### Table 2. Effect of morphine and A. chinensis extract on tail flicking time of mice.

| Groups | 0 min    | 30 min   | 60 min   | 90 min   |
|--------|----------|----------|----------|----------|
| Control (10 ml/kg) | 6.03±0.47 | 6.26±1.36 | 6.40±1.03 | 6.00±0.45 |
| Morphine (Standard) (2 mg/kg) | 6.48±0.69 | 10.80±0.91* | 15.30±1.12** | 10.22±1.37** |
| Methanolic crude extract (200 mg/kg) | 6.80±0.54 | 7.86±0.46 | 8.86±0.47 | 6.00±0.50 |
| Methanolic crude extract (400 mg/kg) | 6.44±0.33 | 9.58±1.56* | 7.82±0.77 | 6.10±0.89 |

Values are expressed as mean ± SD; *p<0.05, **p<0.01.

### Table 3. Anti-nociceptive activity of the crude methanol extract of A. chinensis.

| Groups | 30 min | 60 min | 90 min |
|--------|--------|--------|--------|
| Morphine (standard) (2 mg/kg) | 72.52 | 139.06 | 70.33 |
| Methanolic crude extract (200 mg/kg) | 25.56 | 7.19 | 0.00 |
| Methanolic crude extract (400 mg/kg) | 53.03 | 22.19 | 1.67 |

### Table 4. Effect of the crude methanol extract of A. chinensis on castor oil (1 ml/mice) induced diarrhea in mice.

| Groups | Number of diarrheal faeces | % Reduction of diarrhea |
|--------|---------------------------|------------------------|
| Control (10 ml/kg) | 16.80±3.83 | --- |
| Loperamide (Standard) (50 mg/kg) | 4.80±0.84** | 77.20 |
| Methanolic crude extract (200 mg/kg) | 6.40±0.89** | 61.90 |
| Methanolic crude extract (400 mg/kg) | 5.20±1.10** | 69.05 |

Values are expressed as mean ± SD; **p<0.01.
animal model, luteolin shortened the duration of γ-hydroxybutyrate-induced sleep (Wang et al., 2008). On the other hand, quercetin exhibited antidepressant activity (Paulke et al., 2008).

Conclusion

Traditional medicines are gaining popularity day by day. For many years, people have been depending on plant sources for the cure of different ailments. Moreover, the high price and associated side effects of modern medicines have made general people relying on herbal medicines. In this study, the crude methanol extract of A. chinensis was profiled for peripheral and central analgesic, anti-diarrheal, anti-inflammatory activities and sleep induction property. Until now, many plants that have been used for thousands of years for specific disease have been screened and later specific compounds have been isolated responsible for that activity. Therefore, phytoconstituents responsible for the observed activities should be isolated, characterized and involved in bioassays. A. chinensis is an excellent candidate for future investigation for potent phytocomponents with biological activities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Table 5. Effect of the methanolic crude extract of A. chinensis on carrageenan induced paw edema in Wistar rats.

| Groups                             | Paw circumference before carrageenan injection (cm) | At 1st h after carrageenan injection (cm) | At 4th h after carrageenan injection (cm) | % Inhibition of edema at 4th h after carrageenan injection |
|------------------------------------|-----------------------------------------------------|-------------------------------------------|-------------------------------------------|----------------------------------------------------------------|
| Control (100 ml/kg)                | 1.18±0.09                                           | 1.84±0.11                                 | 2.12±0.05                                 | 0.66                                                             |
| Indomethacin (Standard) (10 mg/kg) | 1.30±0.07                                           | 1.74±0.11                                 | 1.36±0.06                                 | 0.44                                                             |
| Methanolic crude extract (200 mg/kg) | 1.28±0.08                                           | 1.92±0.18                                 | 1.40±0.01                                 | 0.56                                                             |
| Methanolic crude extract (400 mg/kg) | 1.30±0.08                                           | 1.86±0.06                                 | 1.38±0.08                                 | 0.56                                                             |

Values are expressed as mean ± SD; **p<0.01.

Table 6. Effect of the crude methanol extract of A. chinensis on phenobarbitone sodium-induced sleeping time.

| Groups                             | Time of onset of sleep (min) | Total sleeping time (min) |
|------------------------------------|------------------------------|---------------------------|
| Control (10 ml/kg)                 | 16.40±1.34                   | 111.40±1.14               |
| Crude methanol extract (200 mg/kg) | 12.40±1.14                   | 110.20±1.92               |
| Crude methanol extract (400 mg/kg) | 10.40±1.14                   | 101.00±0.71               |

Values are expressed as mean ± SD.
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