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Antinociceptive and Anti-inflammatory Activities of Flower (*Alangium salvifolium*) Extract

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Abstract: The present study aimed to evaluate the antinociceptive and anti-inflammatory activity of *Alangium salvifolium* (AS) flower in mice. The antinociceptive activity was determined using tail immersion, acetic acid induced writhing and formalin induced licking test. Anti-inflammatory effects were evaluated using carrageenan and formalin induced paw edema in mice. The methanol extract (50 and 100 mg kg⁻¹) of flower of AS followed by chloroform extract (100 mg kg⁻¹) produced a significant inhibition of both phases of the formalin pain test in mice, a reduction in mice writhing induced by acetic acid and delayed the response of mice to hot water thermal stimulation in tail immersion test. All flowers extract of AS also produced a substantial inhibition (nearly 50%) of carrageenan and formalin induced paw edema. The inhibitions were similar to those produced by indomethacin, p.o. The different alkaloids and flavonoids found in the extract could be account for the antinociceptive and anti-inflammatory actions.

Key words: *Alangium salvifolium*, antinociceptive, anti-inflammatory, flower, flavonoids

INTRODUCTION

Alangium salvifolium Wang is a deciduous, rambling shrub or a tree belonging to the family Alangiaceae. This family consist one genus with twenty two species, out of which *A. salvifolium* (AS) is the only species used medicinally in Bangladesh, India, China and Phillipines (Jubie *et al.*, 2008). Traditionally there were no specific uses of flower but the plant has been reported for its anti-tubercular, anti-spasmodic and anti-cholinesterase activity (Warrier *et al.*, 2005). Chloroform extract of AS flower showed antibacterial activity that yield two alkaloids alangimaridine and methyl-1H-pyrimidine-2,4-dione (Anjum and Haque, 2003) and one steroid 3-O- β -D-glucopyronosyl-(24 β)-ethylcholesta-5,22,25-triene (Anjum *et al.*, 2002). On the other hand ethanolic extracts of flower possess tannins and polyphenes which exhibit cardiac effect (Venkateshwarlu *et al.*, 2011).

Literature survey reveal that different parts of AS are showed various biological activity specially roots exhibit analgesics and inflammatory effects (Porchezian *et al.*, 2001). Recently Venkateshwarlu *et al.* (2011) and his group reported diverse therapeutic and medicinal applications against various diseases by different parts of this species. However, there is lack of research on biological activity of flower and its chemical characterization. Therefore we were interested to carry out our research work on flower part of this species to

evaluate the analgesic and anti-inflammatory effect along with phytochemical screening.

MATERIALS AND METHODS

Plant materials: The flower of the plant *Alangium salvifolium* (AS) was collected from the Rajshahi, Bangladesh, during February to March 2010. The identity of the plant was confirmed by Botany department, Rajshahi University, Bangladesh. The plant was deposited in the same institution with a voucher specimen no 105 for further correspondence.

Extraction and sample preparation: Shed dried flower of AS powder (750 g) were extracted with 100% MeOH (500 mL) for 7 days and repeated the whole extraction twice. Combined extracts were filtered and evaporated to dryness in vacuo using Rotary evaporator at 40°C to get a semisolid crude mass (approx. 5 g) than chloroform soluble fraction was prepared by solvent-solvent extraction process and the chloroform extract was evaporated to dryness under reduced pressure at 40°C to yield a crude chloroform fraction (approx. 2.3 g).

Anti inflammatory activity

Carrageenan induced paw edema test: Male wistar mice (55-65 g) were divided into five groups of six animals each. The test groups received orally 50 and/or 100 mg kg⁻¹ of

each extract. The reference group received indomethacin (10 mg kg⁻¹, p.o) while the control group received Vehicle (1% tween 80). After 1 h, 0.1 mL, 1% w/v carrageenan suspension in normal saline was injected into the subplantar tissue of the right hind paw (Winter *et al.*, 1962). The paw volume was measured at 1, 2, 3 and 4 h after carrageenan injection using a micrometer screw gauge. The percentage inhibition of the inflammation was calculated from the formula:

$$\text{Inhibition(\%)} = \frac{1-D_t}{D_0} \times 100$$

whereas, D₀ was the average inflammation (hind paw edema) of the control group of rats at a given time, D_t was the average inflammation of the drug treated (i.e., extract/fractions or reference indomethacin) rats at the same time (Gupta *et al.*, 2005).

Formalin induced edema in the mice paw: Formalin 0.02 mL (2.5% in distilled water) was injected into the subplantar area of the right hind paw of mice (Chau, 1989). Samples were given 1 h prior to formalin injection. At 4 h after formalin injection, the paw thickness was determined using a dial thickness gauge to measure the degree of inflammation. At the end of the experiment the animals were killed by cervical dislocation and the paws were cut at the knee and the increase of the weight of the right hind paw versus the left hind paw noted.

Analgesic activity

Tail immersion test: The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice (Toma *et al.*, 2003). From 1-2 cm of the tail of mice was immersed in warm water kept at constant temperature of 60°C. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 sec was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined before and 0, 30, 60 and 90 min after the administration of drugs.

Acetic acid induced writhing test: The analgesic activity of the samples was also studied using acetic acid-induced

writhing model in mice. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac-Na was administered intraperitoneally 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as ‘writhing’ for the next 10 min (Ahmed *et al.*, 2004).

Formalin induced paw licking test: The antinociceptive activity of the drugs was determined using the formalin test described by Dubuisson and Dennis (1977). Control group received 5% formalin. 20 Twenty microlitter of 5% formalin was injected into the dorsal surface of the right hind paw 60 min after administration of extract (50 and/or 100 mg kg⁻¹ of each extract orally) and 30 min after administration of Diclofenac Na (10 mg kg⁻¹, i.p). The mice were observed for 30 min after the injection of formalin, and the amount of time spent licking the injected hind paw was recorded. The first 5 min post formalin injection is referred to as the early phase and the period between 15 and 30 min as the late phase. The total time spent licking or biting the injured paw (pain behavior) was measured with a stop watch.

Statistical analysis: All results are expressed as the mean±SEM. Statistical significance was calculated using student t test by SPSS version 12.0 (SPSS, Inc., Chicago, IL, USA). Values of p<0.05 were considered to indicate statistical significance. All assays were performed in triplicate.

RESULTS AND DISCUSSION

The phytochemical screening of methanol extract of AS showed the extract contains diverse class of chemical constituents (Table 1) such as flavoloids followed by alkaloids, steroids, terpenes etc. In addition, the total polyphenol content and total antioxidant value were measured to be 152.73±13.60 mg g⁻¹ equivalent of ascorbic acid and 803.87±12.11 mg g⁻¹ equivalent of gallic acid, respectively. This is the first report of total polyphenol content and total antioxidant of flower of AS. It is revealed from the results of anti-inflammatory bioassay that methanol extract of AS at 50 mg kg⁻¹ bw exhibit potent anti-inflammatory activity with nearly 50% inhibition (Fig. 1) compared to that of standard (10 mg kg⁻¹ indomethacin, Group-II) after four hours

Table 1: Phytochemical screening of the methanol and chloroform extract of *A. salvifolium* flower

Extract	Carbohydrate	Tannin	Flavonoid	Saponin	Phenol	Steroid	Alkaloid
Methanol	-	++	++	-	++	+	+++
Chloroform	-	+	+++	-	+	++	+++

+: Present; (-): Absent; +++: Reaction intensity is high; ++: Reaction intensity is medium; +: Reaction intensity is normal

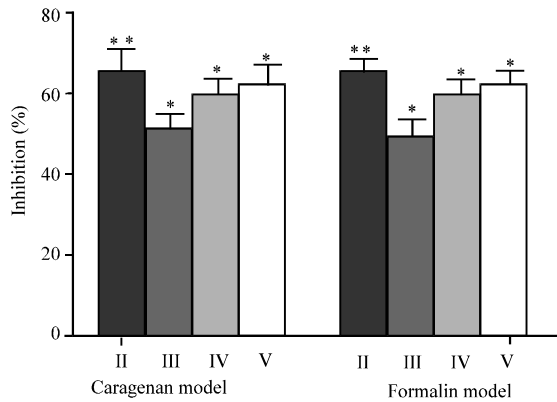


Fig. 1: Inhibition of acute and chronic inflammation by different (G-II, Positive control, 10 mg kg⁻¹ b.wt. G-III, MeOH-50 mg kg⁻¹ b.wt. G-IV, MeOH-100 mg kg⁻¹ b.wt. G-V, CHCl₃-100 mg kg⁻¹ b.wt.) extract of flower of AS. * p<0.005 vs control group

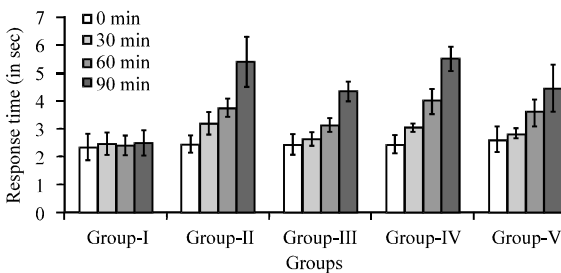


Fig. 2: Effect of different (G-II, Positive control, 10 mg kg⁻¹ b.wt. G-III, MeOH-50 mg kg⁻¹ b.wt. G-IV, MeOH-100 mg kg⁻¹ b.wt. G-V, CHCl₃-100 mg kg⁻¹ b.wt.) extract on tail withdrawal reflex of mice induced by tail immersion method

in both. This is the first report of anti-inflammatory effect of flower of AS by carrageenan and formalin induced paw *in vivo* model. In carrageenan induced model the involvement of cyclooxygenase product of arachidonic acid metabolism and the production of reactive oxygen species are well established (Smith *et al.*, 1974). The results in Fig. 1 show that methanol extract of AS (50 mg kg⁻¹) produced more inhibition of carrageenan induced edema compared with all tested as well as control group. Thus flower of AS could be a good anti-inflammatory agent by inhibiting cyclooxygenase pathway considering that the mechanism involved in the genesis of the carrageenan induced edema could cause the release of prostaglandins and kinins, among other substances (Garcia *et al.*, 1973).

To assess the analgesic activity, flower of AS was evaluated by three screening protocol namely tail immersion, acetic acid induced writhing test and formalin induced paw licking test in mice. As it is shown in Fig. 2, after a latency period of 20 sec following oral administration of methanol extract of AS (50 and 100 mg kg⁻¹) significantly delayed the response of mice to hot water thermal stimulation and it was dose dependant. Similarly chloroform extract of AS exhibited moderate response in mice. The standard drug diclofenac-Na also showed similar response that was highest after 90 min interval of drug administration. As tail immersion method is commonly used for response, suggesting that the methanol extract of AS may used as a central analgesic principles. On the other, hand Acetic acid induced writhing model is a sensitive procedure to evaluate peripherally active analgesic. The methanol extract of AS induced a dose dependent inhibition of writhing (58.06±1.38 at 100 mg kg⁻¹) after acetic acid injection in mice, and this effect (Table 2) was also noted in case of chloroform extract (51.60±1.86 at 100 mg kg⁻¹) after intraperitoneal administration compared with standard drug diclofenac-Na (percentage of inhibition 70.96 ±1.86 at 10 mg kg⁻¹body weight). It is well known that non steroidal anti inflammatory and analgesic drugs mitigate the inflammatory pain by inhibiting the formation of pain mediators at the peripheral target sites where prostaglandins and bradykinin are proposed to play a significant role in the pain process (Hirose *et al.*, 1984).

The most predictive of the models of acute pain is undoubtedly the formalin test (Dubuisson and Dennis, 1977). Intraplantar injection of 5% formalin evoked a characteristic biphasic licking response, an early phase corresponding to acute neurogenic pain, sensitive to drugs that interact with the opioid system and a late phase corresponding to inflammatory pain responses inhibited by analgesic-antiinflammatory drugs (Hunnskaar and Hole, 1987). In the formalin test (Fig. 3), pretreatment of mice with the methanol extract of AS at the doses of 50 (Group- III) and 100 mg kg⁻¹ (Group- IV) had significant effect followed by chloroform extract (Group-V) during the first phase of the test (0-5 min) and the second phase (15-30 min). Indomethacin was used as a positive control in this experiment. Recent studies have shown that the earlier phase of formalin-induced pain reflects the direct effect of formalin on nociceptors, whereas the late phase reflects inflammatory pain, which appears to be attributable to prostaglandin synthesis (Hong and Abbott, 1995). The effect produced in the first phase may be due to immediate and direct effects on sensory receptors, bradykinin receptors or glutamatergic way, whereas for the last phase the

Table 2: Effect of the different (G-II, Control 10 mg kg⁻¹ b.wt. G-III, MeOH-50 mg kg⁻¹ b.wt. G-IV, MeOH-100 mg kg⁻¹ b.wt. G-V, CHCl₃-100 mg kg⁻¹ b.wt.) extract on acetic acid induced mice

Group	Dose (mg kg ⁻¹)	Writhing No.	Inhibition of writhing No (%)
I		30.0±.57	
II	10	9.0±0.57	70.96±.86***
III	50	21.0±1.15	32.25±3.72**
IV	100	13.0±0.57	58.06±1.38***
V	100	15.0±0.57	51.60±1.86***

All values are expressed as mean±SEM, (n = 6); student t test by SPSS version 12.0. ***p and **p<0.001, significant compared to control

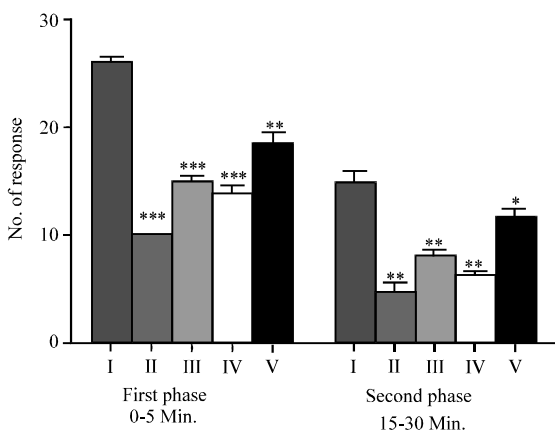


Fig. 3: Effect of different (G-II, Positive control, 10 mg kg⁻¹b.wt. G-III, MeOH-50 mg kg⁻¹ b.wt. G-IV, MeOH-100 mg kg⁻¹ b.wt. G-V, CHCl₃-100 mg kg⁻¹ b.wt) extract on hind paw in the formalin induced licking test in mice. *p<0.005 vs control group

analgesic effect is related to the inflammatory responses induced by arachidonic acid cascade (Dubuisson and Dennis, 1977; Bodi and Nodine, 1964; De Souza *et al.*, 1998). The ability of methanol extract of AS to have an effect on both phases indicates that it contains an active analgesic principle acting both centrally and peripherally. However, this is the first time this study demonstrated that the methanol extract of AS possess antinociceptive effect through three different methods i.e tail immersion, acetic acid and formalin induced in mice.

Taken all together, this is an indication that the extract of flower of AS can be used to manage acute as well as chronic pain. However, Kerstein *et al.* (2009) suggest that, the inhibitory effect to pain response is due to inhibit the increase of the intracellular Ca²⁺ ion through the TRPA1 (Transient Receptor Potential Ankyrin 1), a member of the transient receptor potential family of cation channel that trigger the analgesic action. So it is likely that the flower extract of AS may contain substances that affect the metabolism of Ca²⁺ ions. On the other hand, preliminary phytochemical screening asserts that flower extract of AS possess alkaloids, flavonoids, steroids, tannins etc. Literature survey revealed that the AS is a

good source of alkaloids and flavonoids (Venkateshwarlu *et al.*, 2011) and especially the flower of AS yield two alkaloids and one steroid that we mentioned previously. In the last decades a large number of different kinds of naturally occurring alkaloids with antinociceptive activity have been reported (Calixto *et al.*, 2000).

Therefore it could be suggested that antinociceptive effect of the flower extract may be due to the content of alkaloids. In addition, flavonoids have been found to suppress the intracellular Ca²⁺ in a dose dependant manner, as well as the release of pro inflammatory mediators such as TNF α (Kempuraj *et al.*, 2005). Recently Annegowda *et al.* (2010) reported that flavonoids may increase the amount of endogenous serotonin and may interact with 5-HT₂A and 5-HT₃ receptors which may be involve in the mechanism of central analgesic activity. There are also reports on the role of flavonoid, a powerful antioxidant (Vinson *et al.*, 1995; Brown and Rice-Evans 1998), in analgesic activity primarily by targeting prostaglandins (Ramesh *et al.*, 1998; Narayana *et al.*, 2001). Tannins are also found to have a contribution in antinociceptive activity (Ramprasad *et al.*, 2006). So it can be assume that cyclooxygenase (COX) inhibitory activity along with antioxidant activity may reduce the production of free arachidonic acid from phospholipid or may inhibit the enzyme system responsible for the synthesis of prostaglandins and ultimately relieve pain sensation.

In conclusion, the flower extract of AS significantly antagonized acetic acid induced writhing, tail immersion and significantly attenuated the nociception produced by formalin as well as reducing the inflammation induced by carrageenan and formalin. The exact mechanism of action and the active principles responsible for antinociceptive and/or anti-inflammatory activities remain to be confirmed and currently isolation of active principles and underlining the mechanism of action is going on in our lab.

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