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Antinociceptive and Anti-Inflammatory Properties of the Methanol Leaf Extract of *Argyreia argentea*

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Abstract:
The present study was carried out to investigate the antinociceptive and anti-inflammatory properties of methanol leaf extract of *Argyreia argentea*. Antinociceptive properties of the extract were evaluated on the pain induced by acetic acid and formalin in Swiss albino mice. The extract at a dose of 1, 1.5 and 2 gkg^{-1} produced an inhibition of 15.81%, 52.91% and 58.71% on acetic acid induced pain and an inhibition of 29.56%, 39.51% and 45.96% on formalin induced pain. Oral administration of the extract significantly ($P<0.001$ and $P<0.01$) reduced writhing response induced by acetic acid and licking response induced by formalin. The anti-inflammatory activity of the same extract was estimated by measuring the mean increase in hind paw volume of carrageenan-induced Wistar albino rat with the help of plethysmometer. Oral administration of extract at a dose of 0.5, 1.0 and 1.5 gkg^{-1} showed the highest inhibition 25.92%, 37.03%, and 34.57%, respectively at the 4th hour of administration. *Argyreia argentea* extract showed significant ($P<0.001$ and $P<0.01$) effect in the reduction of the paw edema induced by carrageenan. The degree of anti-inflammatory and antinociceptive activity of extract was compared to the effect of standard drug diclofenac sodium. Morphine is used as a standard drug in formalin induced licking response model. In acute toxicity study, no mortality was observed at 4 gkg^{-1} dose level. The present study demonstrates the potential anti-inflammatory and analgesic effect of the methanol leaf extract of *Argyreia argentea* which supports the claims by the traditional medicine practitioners.
Key Words: A*rgyreia argentea;* Anti-inflammatory; Antinociceptive; formalin and Morphine.

Introduction:

Argyreia argentea is an evergreen shrub that is mainly found in Chittagong Jessore, Mymensingh, Noakhali, Sylhet and Tangail of Bangladesh and it is widely distributed in Eastern India, Bhutan and Nepal [1]. It is widely used by the tribal communities of Chittagong Hill tracts for the treatment of various diseases. The plant is locally known as Bitarak Rupar tola ludi by Chakma and Kajinganj, Naiprabong by Marma of Bangladeshi tribal communities. The plant grows in secondary forest and scrub jungles. It gives flower and fruit during July to October. The plant is used in the treatment of Boils, Gastric, Tumour, Marasmus, Paralysis and Spermaforrhoea [1]. Another important species of *Argyreia* is *A. nervosa* locally known as Elephant creeper. Its root and leaves are used in the treatment of nervous disorder, skin infection, gonorrhea and aphrodisiac.

Despite the traditional use of *A. argentea* since long in the rheumatoid arthritis and cold, no scientific work has been done to ascertain its analgesic and anti-inflammatory activity albeit the antipyretic effect of petroleum ether and chloroform soluble fractions of ethanol extract of its roots has been observed recently [2]. Present study aims to evaluate the antinociceptive and anti-inflammatory properties of *A. argentea* methanol leaf extract in animal model.

Materials and Methods:

Collection of plant

The plant leaves were collected from Chittagong district, Bangladesh. The plant was taxonomically identified by Dr. Shaikh Boktear Uddin (Associate Professor, Department of Botany, University of Chittagong, Bangladesh). A voucher specimen (accession no is 34198) has been deposited at the Bangladesh National Herbarium, Mirpur, Dhaka.

Preparation of plant extract

The fresh leaves of *A. argentea* were washed with distilled water, chopped into small pieces, and air dried at room temperature (24±5) 0C for about 10 days to grind into powder. The resulting leaf powder (500g) was extracted in Erlenmeyer flasks with cold methanol being stirred and macerated at room temperature (23±5)^{0}C for 15 days at room temperature. The methanol was

evaporated under reduced pressure below 50^0C through rotatory vaccum evaporator (RE200 Sterling, UK). The concentrated stem extract (25g blackish-green) was stored at 4^0C until use.

Experimental Animals and Diets

Swiss albino mice of both sexes weighing between 25 to 30 gm and Wistar Albino rats of the either sex weighing between 150-200 gm obtained from animal house of Bangladesh Council for Scientific and Industrial Research (BCSIR) laboratories, Chittagong were used for present study. The animals were acclimatized to room temperature $(26\pm5)^0$C with a relative humidity of 55±5% in a standard wire meshed plastic cages for 4 to 5 days prior to commencement of the experiment. During the entire period of study the animals were supplied standard pellet diet and water *ad libitum.* All animal experimentations were carried out with the guidelines of Institutional Animal Ethics Committee (IAEC).

Assay for antinociceptive activity

Acetic acid induced writhing test

The abdominal constriction was induced in mice (weighing 25-30 gm) by intraperitoneal injection of 1% (v/v) acetic acid (2.3 ml kg^{-1}), as described by Koster *et al*. [3]. Animals were pre-treated with the methanol extract of *A. argentea* (1, 1.5 and 2g kg^{-1}), 30min before acetic acid administration. Control animals received a 2ml volume of distilled water and the positive control animals were treated with reference analgesic drug diclofenac sodium (40 $mgkg^{-1}$). The number of abdominal constrictions was cumulatively counted over a period of 20 min. The percentage inhibition of analgesic activity was calculated by using following formula-

$$\% \text{ Analgesic activity} = \frac{\text{Mean writhing count (Control group- Treated group) x100}}{\text{Mean writhing count of control group}}$$

Formalin test

The procedure was similar to that described previously by Gaertner *et al.* in 1999 [4]. 20 µL of 2.5% formalin (0.92% formaldehyde) made in phosphate buffer was injected under the right hind paw surface of experimental mice. Animals were pre-treated with the methanol extract of *A. argentea* (1, 1.5 and 2 gkg^{-1}). Each mouse was placed individually in a cage and observed from 0 to 5 min followed by the injection of formalin to analyze the first phase of formalin induced pain (neurogenic pain). The length of time the animal spent licking the injected paw was timed with a chronometer and was considered as indicative of pain.

Assay for anti-inflammatory activity

Anti-inflammatory activity of *A. argentea* extract was assessed by using carrageenan induced paw edema model of rat by the reported method of Winter *et al*. [5]. According to Winter, acute inflammation (paw edema) was induced in albino rats by subplantar injection of 100µl of 1 % (w/v) carrageenan after measuring the initial right hind paw volume of each rat. The volume of right hind paw was measured at 1^{st}, 2^{nd}, 3^{rd} and 4^{th} hour after carrageenan injection and the paw edema was determined using plethysmometer (7150 UCG Basil, Italy). *A. argentea* stem extract (0.5, 1.0 and 1.5 gkg^{-1}), standard anti-inflammatory drug diclofenac sodium (40 $mgkg^{-1}$), and distilled water were administered orally to treated, positive control and control groups one hour before the subplantar injection of carrageenan. The paw edema was determined using the following formula; Edema = final volume - initial volume.

Acute toxicity studies

Methanol leaf extract of *A. argentea* was injected intraperitoneally in mice at various dose levels namely 500 $mgkg^{-1}$, 1 gkg^{-1}, 2 gkg^{-1}, 3 gkg^{-1} and 4 gkg^{-1} of body weight. Five mice in each dose group were closely observed for 24 hours for any mortality and next ten days for any delayed toxic effect.

Statistical analysis

Values for analgesic activity were expressed as "mean increase in latency after drug administration ±SEM" in terms of seconds whereas values for anti-inflammatory activity were expressed as "mean increase in paw volume ±SEM". The significance of difference between means was determined by student's t-test values of $p<0.05$ were considered significant and $p<0.01$ and $P<0.001$ as highly significant.

Results:

The results obtained with acetic acid-induced writhing are shown in Table 1. All doses administered (1, 1.5 and 2.0 g kg^{-1}) had a significant ($P<0.001$ and $P<0.01$) effect on the number of abdominal constrictions, promoting 15.81%, 52.91% and 58.71% inhibition, respectively, as compared with the control group treated with distilled water. These degrees of inhibitions were comparable to standard analgesic drug diclofenac sodium (72.57%). The methanol extract asserted a significant analgesic action against early phase of formalin induced pain. The methanol extract at 2 gkg^{-1}, 1.5 gkg^{-1} and 1 gkg^{-1} inhibited the effect of formalin induced pain by 45.96%, 39.51% and 29.56%, respectively. On the other hand the drug morphine at 0.5mg/kg inhibited the effect of formalin induced pain by 50% (Table 2).

As shown in Table 3, sub-plantar injection of carrageenan in rats showed a time-dependent increase in paw thickness which was observed at 1 h and was maximal at 4h after the administration of carrageenan injection in the control group. The anti-inflammatory effects of *A. argentea* leaf extract in carrageen induced hind paw edema are presented in Table 4. The extract 1.5 gkg^{-1} produced 25%, 25.88%, 32.29% and 34.56% effect at 1h, 2h, 3h and 4h, respectively and 1 gkg^{-1} produced 17.5%, 17.64%, 19.79%, and 37.03% effect at 1h, 2h, 3h and 4h, respectively whereas 0.5 gkg^{-1} produced 10.58%, 13.54% and 25.92% effect at 2h, 3h and 4h, respectively after carrageenan injection (Table 4) where significant values are marked with asterisk.

Discussion:

According to our findings, the methanol extract of *A. argentea* leaf produced an antinociceptive effect when assessed in chemical models of nociception, including acetic acid-induced writhing and formalin tests. In acetic acid-induced writhing, a dose-dependent antinociceptive effect of the extract was observed. It is proposed that the acetic acid acts indirectly by inducing the release of endogenous mediators which stimulate the nociceptive neurons sensitive to non-steroidal anti-inflammatory drugs (NSAIDs) and opioids [6].

Acid acetic-induced abdominal constrictions are useful experimental tool in the testing of new analgesic drugs [7] since the abdominal injection of acetic acid in mice has been attributed to the release of arachidonic acid, which results the synthesis of prostaglandin via the cyclooxygenase (COX) enzyme [8]. The special nerve endings that sense pain are very sensitive to prostaglandin. When prostaglandin is released, the nerve endings respond to it through prostaglandin E2 (PGE2) receptor by picking up and transmitting the pain and injury messages through the nervous system to the brain and cause visceral writhing stimuli in mice [9-11]. Therefore, it has been suggested that the inhibition of prostaglandin synthesis is remarkably efficient as an anti-nociceptive mechanism in visceral pain [12]. Since, methanol extract of *A. argentea* showed

Table 1: Effect of *A. argentea* stem extract on acetic acid-induced writhing response in mice

Groups (n=6)	Writhing Response	Analgesic Percent
Control	51.67 ± 2.12	-
AAEx 1.0gkg^{-1}	43.50 ± 1.176*	15.81%
AAEx 1.5gkg^{-1}	24.33 ± 1.35**	52.91%
AAEx 2.0gkg^{-1}	21.33 ± 1.22**	58.71%
DS	14.17± 1.56**	72.57%

AAEx: *Argeria argentea* extract; DS, Diclofenac sodium 40mgkg; All values of writhing responses are expressed as mean ± SEM (n=6); Degree of freedom is 10 in all cases; *** P < 0.001, ** P < 0.01 and * P<0.05 significant compared to control (Student's t- test).

Table 2: Effect of *A. argentea* stem extract and morphine in % reduction of licking response.

Treatment	Licking time	Inhibition %
Control	74.4±1.157	-
AAEx 1.0 g/kg	52.4±1.44*	29.56
AAEx 1.5 g/kg	45.0±2.0*	39.51
AAEx 2.0 g/kg	40.2±1.53*	45.96
Morphine 0.5 mg/kg	39.0±0.75*	50.00

All values are expressed as mean ± SEM (n=5); Degree of freedom is 8 in all cases; *P<0.001 significant compared to control in all cases

Table 3: Evaluation of anti-inflammatory activity of *A. argentia* leaf extract by carrageenan induced paw edema model

Group (n=10)	Hind Paw edema (mm^3)			
	1h	2h	3h	4h
Control	0.4±0.007	0.85±0.014	0.96±0.008	0.81±0.005
AAEx 0.5gkg^{-1}	_	0.76±0.014**	0.83±0.005*	0.6±0.021*
AAEx 1gkg^{-1}	0.33±0.010*	0.7±0.028**	0.77±0.003*	0.51±0.002*
AAEx 1.5gkg^{-1}	0.3±0.009*	0.63±0.016*	0.65±0.004*	0.53±0.01*
DS	0.24±0.003*	0.49±0.007*	0.47±0.002*	0.37±0.005*

All values are expressed as mean ± SEM (n=5); Degree of freedom is 8 in all cases; *P<0.001 significant compared to control; **P<0.01 significant compared to control

significant (P<0.001 and P<0.01) inhibition of acetic acid induced writing response of mice, it can be suggested that the leaf extract has potential analgesic activity. Though the exact mechanism of action behind this effect is yet not clear, it can be suggested that this effect may be due to inhibition of the synthesis of arachidonic acid metabolites [13].

The formalin test which is sensitive for various classes of analgesic drugs has two distinct phases, reflecting different types of pain. The early phase (initial pain) reflects a direct effect of formalin on nociceptors (neurogenic pain) whereas the late phase reflects tissue injury or inflammatory pain **[**14**,** 15]. In the formalin test, several mediators such as histamine, kinin, serotonin and prostaglandins are released from damaged cells which take part in the inflammatory response and are able to stimulate nociceptors and induction of pain

Table 4: Effect of *A. argentea* leaf extract in the inhibition of paw edema in mice

Group (n=10)	% Inhibition of paw edema $\frac{(Ct - Co)\ control - (Ct - Co)\ treated}{(Ct - Co)\ control} \times 100$			
	1st hr	**2nd hr**	**3rd hr**	**4th hr**
Control (Dis. H_2O)	-	-	-	-
AAEx 0.5gkg^{-1}	_	10.58%	13.54%	25.92%
AAEx 1.0gkg^{-1}	17.5%	17.64%	19.79%	37.03%
AAEx 1.5gkg^{-1}	25%	25.88%	32.29%	34.57%
Positive control (DS)	40%	42.35%	51.04%	54.32%

Co is the paw thickness volume (mm^3) before carrageenan injection, Ct is the paw thickness volume (mm^3) at time t, (Ct -Co) is paw edema.

[16]. In this test, the centrally acting drugs such as narcotics inhibited both phases equally, while peripherally acting drugs only inhibited the second phase [17]. It is also well known that the formalin model may involve sensorial C-fibers [18] in early phase and a combined process generated by peripheral inflammatory tissue and functional changes in the dorsal horn in late phase [19**,** 20]. Our results showed that the time spent in licking and biting of the injected paw was significantly ($P<0.001$) reduced by intraperitoneal administration of the methanol extract of *A. argentea*, in both phases. In fact, the effect of the extract on both phases showed that they contain active analgesic principles acting both centrally and peripherally.

Morphine was used as a standard and its pain reduction activity was 50%. Morphine, the prototypical opioid analgesic, is metabolized in vivo primarily to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). These metabolic products account for ~65% of a dose of morphine, with the remaining drug biotransformed to multiple minor species or excreted unchanged. This finding indicated that opioid system at least partially involves in antinociception action. Intraplantary injection of formalin may be activated the endogenous micro-opioid system and exerts a tonic inhibitory effect on the pain behaviors [21]. This probably suggests or indicates that the extract exerts its analgesic effect through both peripheral inhibitory actions and released prostaglandins (inflammatory pain) and central activity relates to antagonistic action of the nociceptors (neurogenic pain) [22].

Few plant metabolites such as flavonoids are known for their antinociceptive and /or anti-inflammatory activity [23-25]. The inhibitory effects of extract in our study may be due to the presence of such components as the qualitative experiment in our lab showed that flavonoids are present in this plant. Modulatory effect on peripheral antinociception may also be induced by this metabolite. However, the results of the present study demonstrate that the methanol extract of *A. argentea* has a significant effect against pain in the current antinociceptive models in mice.

Several inflammatory mediators like complement, histamine, kinins, prostaglandins and pro-inflammatory cytokines have been suggested to play a role

in the mechanism of inflammation [26, 27]. It is assumed that at least some of these mediators are subjects of inhibition by the methanol extract of *A. argentea.* Carrageenan-induced paw edema is suitable for screening anti-inflammatory properties for natural drugs because of its sensitivity in detecting orally active anti-inflammatory (anti-edematous) agents particularly in the acute phase of inflammation [28, 26]. Development of edema in the paw of rat after injection of carrageenan is a biphasic event [29]. The initial phase observed during the first hour is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease, and lysosome [30-32]. This leads to a dilation of the arterioles and venules and to an increased vascular permeability. As a consequence, fluid and plasma proteins are extravasated, and edema forms [33]. The mediators, including histamine, 5-HT, the kinins and their complements, have become the recent focus of attention as the metabolites of arachidonic acid (AA). Alone or in appropriate combination, AA products of COX pathway are capable of producing the characteristic signs of inflammation: vasodilatation, hyperemia, pain, edema, and cellular filtration. The COX products, particularly prostaglandin E2 (PGE2), contribute to increased blood flow through a vasodilatation action, but the lipoxygenase (LOX) pathway is necessary for vascular leakage and edema consequently on cellular infiltration.

Oral administration of methanol extract of *A. argentea* showed the significant ($P<0.001$ and $P<0.01$) dose dependent anti-inflammatory activity in carrageenan induced hind paw edema in rats. These results support scientifically the use of *A. argentea* in popular folklore medicine. Since crude methanol extracts of *A. argentea* showed significant anti-inflammatory and analgesic properties, we assume that different active secondary metabolites are present in crude extracts and perhaps some of these compounds may operate in a synergistic manner.

In the acute toxicity assay of ethanol extract of *A. argentea,* no death of mice was observed at higher (4 g/kg) dose level. The doses did not show any abnormalities.

Conclusion:

To summarize, the methanol extract of leaf from *A.argentea* possesses analgesic effect against chemical models of nociception while thermal models are unstudied. This action is either central or peripheral, but the exact mechanism is still in question. Indeed, the extract presents clear anti-inflammatory effects. Some secondary metabolites can be related, at least in part, to the analgesic and anti-inflammatory effects of methanol extract of leaf of *A. argentea.* Further studies, obviously, will be inevitable to understand the mechanisms of action underlying the effects of the extract and their active compounds. However, this is the first scientific basis to use the plant as a good source of analgesic and anti-inflammatory action.

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