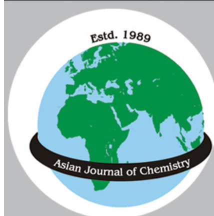

Asian Journal of Chemistry; Vol. 24, No. 11 (2012), 4995-4999

ASIAN JOURNAL OF CHEMISTRY

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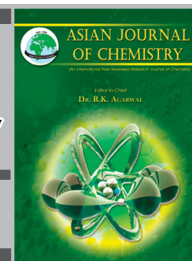

Studies on Antiinflammatory, Antipyretic and Analgesic Activities of *Aporusa oblonga* Mull Arg

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(*Received*: 9 September 2011; *Accepted*: 6 June 2012) AJC-11545

Aporusa oblonga Mull. Arg. (Euphorbiaceae) is a well known for its medicinal and traditional use in Mizoram (north eastern part of India). It is commonly used by local folklores for the treatment of various ailments which include stomach ache, ulcer, diarrhoea and dysentery. However, to be clinically useful, more scientific data are needed. Therefore, in the present study, we investigated the effect of *Aporusa oblonga* on acute and chronic inflammation and by thermally induced pain stimulus for its antiinflammatory and analgesic activities in animal models. To assess the antiinflammatory and analgesic properties varied concentrations of methanolic extract of *Aporusa oblonga* (100, 200, 400 mg/kg, orally) were tested in carrageenan-induced rat paw oedema, cotton pellet granuloma, brewer's yeast induced pyrexia, acetic induced writhing, formalin test, tail flick test and hot plate reaction time in experimental rats. The paw volume in experimental rats were reduced significantly ($p < 0.05$) as compared to that of control and hot plate test showed significant (jumping) effect in rats. These results clearly indicate that the stem bark of *Aporusa oblonga* used traditionally by people in Mizoram could be a potential source for using as antiinflammatory and analgesic agent.

Key Words: *Aporusa oblonga*, Analgesic, Antiinflammatory.

INTRODUCTION

The plant *Aporusa oblonga* is a tree which belongs to family Euphorbiaceae. It is a commonly occurring in the tropical valleys of the Eastern Himalayas (North Eastern India) and hills of Assam, Meghalaya, Arunachal Pradesh and in Odisha[1]. It is also found in North India and Pakistan southwards to Burma, Thailand, Laos, Vietnam, South China, Cambodia, Nicobar Island and Malaysia. The young leaves are taken as vegetable. The infused filtrates of the bark are usually used by the local folklore as a remedy for stomach/intestinal pain, ulcer, diarrhoea and dysentery[2]. Thus based on the above facts, we hypothesized that the methanolic extract of the stem bark of *A. oblonga* (MAO) may have potential analgesic and antiinflammatory activity.

A. oblonga is traditionally used by the local folklores of Mizoram, however no chemical constituents of the plant has earlier been reported. As the traditional uses of the stem bark of *A. oblonga* have not been scientifically evaluated, the present study was designed to evaluate the antiinflammatory and antipyretic effects of methanolic extract of the stem bark of *A. oblonga* in rats and rabbits, respectively.

EXPERIMENTAL

A. oblonga (*Euphorbiaceae*) was collected from the rural belt of Thingdawl, Kolasib (Mizoram) in September 2008 and was identified by the taxonomist at the Botanical Survey of India, Shibpur, Howrah, India. A voucher specimen is deposited in Department of Pharmacy, Regional Institute of Paramedical and Nursing Sciences, Mizoram. The stem bark of the plant was air-dried under shade for 2 weeks at room temperature. The dried plant material was then chopped, pulverized and stored in polyethylene bags under refrigeration until further investigation.

Plant material extraction: Dried and powdered stem bark (2.5 kg) were extracted with continuous Soxhlet extractor using methanol as the solvent for 36 h, filtered and concentrated to give a reddish brown coloured dry extract (48.57 g, 1.93 %).

Animals: Male Wistar albino rats (120-180 g) and adult New Zealand white rabbits (2.3-3.5 kg) of both sexes were maintained at the Animal House of RIPANS, under standard environmental condition of temperature (25 ºC), fed with standard diets and water *ad libitum*.

Drugs and Chemicals: Carrageenen from Sd-fine Chemicals, Mumbai, sodium carboxymethyl cellulose from LOBA Chemmie. Pvt. Ltd. Dichlofenac sodium, Indomethacin, Aspirin from Torent Pharmaceutical Ltd, Ahmedabad, Pethidine injection was obtained from Bengal Immunity, Kolkata. Other Chemicals used were of Analytical Grade from Loba or Merck Company.

Phytochemical analysis

Preliminary chemical tests: Phytochemical properties of the extract were tested as follows: anthraquinones Borntrager's reaction: 2 mL 10 % HCl and 10 % $FeCl_3$ solution + 2 mL of extract, boiled for 10 min. Extract filtered hot, cooled and extracted with 5 mL of chloroform. Chloroform layer tested with 2 mL of dilute NH_3 solution, no coloured precipitate was formed which indicated the absence of free anthraquinone). Tannins (2 mL extract + 10 mL of distilled water, filtered). A 2 mL filtrate + 2 mL $FeCl_3$, no blue-black precipitate indicated the absence of tannins. Alkaloids (2 mL extract + 1 % aq HCl + steam, l mL filtrate + 6 drops of Dragendorrfs reagents. No orange red precipitate indicated the absence of alkaloids). Saponins (frothing test: 0.5 g extract + 5 mL warm distilled water. Frothing persistence meant saponin present). Flavonoids (1 mL filtrate + 1 mL dil NaOH; golden yellow precipitate means flavonoids present). Glycosides (0.5 g extract + 10 mL distilled water and filtered. 5 mL of Fehling's solution A and B added. presence of red precipitate meant presence of glycosides). Steroids (10 mg extract dissolved in 1 mL of chloroform and 2 mL Libermann- Burchard reagent gave Reddish purple colour which indicates presence of steroids)[3].

Antinociceptive activity

Acetic acid writhing reflex: Acetic acid-induced writhing model was employed to evaluate the analgesic activity. It was performed according to Gaertner *et al*[4]. Albino rats (five per group) were injected intraperitoneally with 0.6 % acetic acid at a dose of 10 mL/kg. The extract (100, 200 and 400 mg/kg, p.o.), Aspirin (100 mg/kg, p.o.) and 0.5 % CMC p.o. were administered 0.5 h prior to treatment with acetic acid. The writhings induced by the acid, consisting of abdominal constrictions and hind limbs stretching, were counted for 0.5 h after a latency period of 5 min. Percentage inhibition was calculated using the following formula.

$$\text{Inhibition}\ (\%) = \left\{\frac{(\text{CM} - \text{TM})}{\text{CM}} \times 100\right\}$$

CM: Control mean, TM: treated mean.

Formalin-induced pain: Pain was induced by injecting formalin 0.05 mL of 2.5 % formalin (40 % formaldehyde) in distilled water in the subplantar of the right hind paw. Rats (five per group) were given extract (100, 200 and 400 mg/kg, p.o.), pethidine (5 mg/kg, s.c.) and 0.5 % CMC p.o. 0.5 h prior to injecting formalin. The amount of time licking and biting the injected paw was indicative of pain and was recorded in 0-5 min (early phase) and 15-30 min (late phase)[5].

Tail flick method: Rupniak *et al*[6]. method was followed for the tail flick test with no modifications. The test was evoked by a source of radiant heat, which was focused on the dorsal surface of the tail. Male, healthy rats were examined for latency to withdraw their tails from a noxious thermal stimulus using a tail-flick meter (Ugo Basile 7140, Italy, tail flick apparatus n = 5 for each group). Each rat was tested twice before the administration of the extract and the reaction times were averaged to obtain a baseline. The intensity of heat stimulus was adjusted to achieve a mean tail-flick latency of 3-4 s in control animals. Each rat was then tested 30, 60, 90, 105, 120, 150 and 180 min after the oral administration of 100, 200 and 400 mg/kg of methanolic extract of *A. oblonga*. Control rats received 0.5 % CMC p.o. instead of extract. Light tail-flick test was repeated with 5 mg/kg, s.c. Pethidine administration. Treatments were terminated if the animal did not respond within 15 s in order to avoid tissue damage.

Hot plate method: Hot Plate latency assay was carried according to the method of Eddy *et al*[7]. The rats used for this study were divided into 5 groups. Three groups received the extract (100, 200 and 400 mg/kg) orally while the remaining two groups received control (0.5 % CMC p.o.) and Pethidine (5 mg/kg, s.c.). The animals were each placed on a hot plate maintained at 55 ºC, 0.5 h after administration of the extract, control or pethidine. The time taken for the rats to respond to the thermal stimulus (usually by jumping) was noted as the latency (in sec). The effect of the extract, pethidine and control were also determined after 60 and 90 min of administration to rats.

Antiinflammatory activity

Carrageenan-induced oedema test: 5 groups of five animals per group were used and MAO was administered orally at 100, 200, 400 mg/kg and Diclofenac sodium (75 mg/kg orally) as reference agent. Control rats received 0.5 % CMC p.o. The administration of extract and drugs was 0.5 h prior to injection of 0.05 mL, 1 % carrregeenan in the left hind paw subplanter of each rat[8,9]. The contralateral paw was injected with 0.05 mL saline used as control. Paw volume was measured by Plethysmometrically using Water Plethysmometer (Ugo Basile 7140 Plethysmometer, Italy) at an interval of 1 h upto 4 h after the injection of the carrageenan into the plantar of the left hand paw[10]. Differences in paw volume between the left and right hind paw were taken as measure of oedema.

Cotton pellet granuloma: The method of Mossa *et al*[10]. was used for this study. This involves surgical insertion of sterilized cotton pellet (10 mg in weight) subcutaneously into the groin of rats using ether as anesthetic agent. The rats used for this study were divided into five groups, three groups received the extracts (100, 200 and 400 mg/kg), while the remaining two groups received 0.5 % CMC (control) and Indomethacin (5 mg/kg). After the surgical insertion of a pellet into the groin of rats, the extracts (100, 200 and 400 mg/kg), saline and Indomethacin (5 mg/kg) were administered to the respective groups of the animals for 7 consecutive days. All the animals were sacrificed on the eighth day with an overdose of ether. The pellet and the granuloma were dissected out carefully and dried overnight in an oven at 60 ºC to a constant weight. The weight of the granuloma tissue was obtained by determining the difference between the initial (10 mg) and the final weight of the cotton pellet with its attached granulomatous tissue. The mean weight of the granuloma tissue formed in each group and the percentage inhibition were determined.

Antipyretic activity: The test was performed according to Lu *et al.*[11] on rabbits of either sex. An aliquot of 3 mL/kg of 10 % yeast suspension was subcutaneously injected into the rabbit back. After 5 h, animals showing at least an increase of 1 ºC of rectal temperature were selected for the experiment. The test animals were administered with either vehicle (0.5 % CMC), paracetamol (100 mg/kg), extracts (100, 200 and 400

mg/kg), orally. The rectal temperature was measured at an interval of 1 h upto 5 h after treatment using digital telethermometer (Ugo basil, 7140, Italy).

RESULTS AND DISCUSSION

Phytochemical tests: The crude extract was found to be positive for the presence of steroids, saponins and flavonoids.

Acetic acid writhing reflex: The extracts of MAO 100, 200 and 400 mg/kg, p.o. significantly reduced the acetic acid-induced writhing by 33.77, 60.92 and 76.16 %, respectively (Table-1). The activity was comparable to that of acetyl salicylic acid (100 mg/kg, p.o.).

TABLE-1
EFFECT OF *Aporusa oblonga* ON MOUSE WRITHING REFLEX INDUCED BY ACETIC ACID

Treatment	Dosage (mg/kg)	Number of writhing per 30 min	Inhibition (%)
Control	5 mL/kg	30.2 ± 0.8602	0
Aspirin	100	12.4 ± 0.9274*	58.94
MAO	100	20 ± 0.7071*	33.77
MAO	200	11.8 ± 0.8602*	60.92
MAO	400	7.2 ± 0.6633*	76.16

Values are expressed as mean ± SEM (n = 5). Statistically significant from control group: *$p < 0.01$, Dunnett test.

Formalin-induced pain: The MAO extracts showed a significant ($p < 0.05$) inhibition of the formalin noxious stimulation on both early and late phases of pain at doses of 100, 200 and 400 mg/kg, respectively, in rats (Table-2).

TABLE-2
EFFECT OF *Aporusa oblonga* ON FORMALIN-INDUCED PAW LICKING IN RATS

Group	Licking time[a] (s)		
	Dosage (mg/kg p.o)	0-5 min	15-30 min
Control	0	89.4 ± 0.5099	84.2 ± 0.5831
Pethidine	5	58 ± 0.7071*	54.4 ± 0.7483*
MAO	100	85.4 ± 0.6782*	80.60 ± 0.5099*
MAO	200	71.8 ± 0.5831*	63.8 ± 0.8602*
MAO	400	65.20 ± 0.7348*	60 ± 0.7071*

Values are expressed as mean ± SEM (n = 5). Statistically significant from control group: *$p < 0.01$; Dunnett test.

Tail Flick method: Oral administration of the MAO extract (100, 200 and 400 mg/kg) reduced the number of tail flicks as shown in Table-3 thus the extract produced significant analgesic activity.

Hot Plate method: The methanolic extract of *Aporusa oblonga*. shows significant antinociceptive effect in hot plate method (Table-4).

Carrageenan-induced oedema test: In this model all extract showed significant inhibitory effect on the oedema formation at the 3 h after carrageenan injection was administered as shown in the Table-5.

Cotton pellet granuloma: The result of the cotton pellet granuloma test showed that the extract may significantly ($p < 0.01$) reduce leukocyte migration during the process of inflammation, since this test access the ability of agents to reduce leukocyte infiltration and granuloma formation in the area of inflammation[12,13] (Table-6).

Antipyretic Activity: The results of the antipyretic study showed that intraperitoneal administration of the plant extract at 100 and 200 mg/kg caused a significant ($p < 0.05$) while 200 mg/kg showed very significant ($p < 0.01$) inhibition of the pyrexia induced by yeast (Table-7). The antipyretic effect of 200 mg/kg plant extract was comparable to that of Paracetamol (100 mg/kg) between 2 and 3 h after treatment, significantly reversed hyperthermia.

This study has investigated the scientific reasons behind the folkloric use of *Aporusa oblonga Mull*. in the management of painful conditions and pyrexia. The results indicated that the methanol stem bark extract of the plant was active against all the experimentally induced laboratory models of pain. The relatively high oral median lethal dose (LD_{50}) in mice suggests that the extract is relatively non toxic when taken orally[14]. Acetic acid-induced writhes is a sensitive procedure in detecting analgesic effect of medicinal agents[15]. This pain mechanism is believed to involve, in part, local peritoneal receptors caused by peritoneal fluid concentration of PGE_2 and PGF_2[16] as well as lipoxygenase products by some researchers[17,18]. Therefore, the result of the acetic acid-induced writhing strongly suggests that the mechanism of action may be linked partly to lipoxygenases and/or cyclo-oxygenases. Substances and drugs that produce strong inhibitory effects in the hot plate test can inhibit centrally induced pain and they act as strong analgesic[19,20]. Formalin test is a well established valid model for the study of central sensitization events at the spinal level after peripheral inflammatory state[21]. The two distinct phases in formalin test are due to direct effect of formalin on nociception and due to inflammation with the release of serotonin, histamine, bradykinin and prostaglandins and at least to some degree, the sensitization of central nociceptive neurons[22,23]. Stimulation of opioids receptors has also been suggested as a possible mechanism of action against neurogenic pain[4]. The ability of the extract to inhibit the neurogenic phase suggests that it possesses central analgesic activity. Carrageenan-induced hind paw oedema is the standard experimental model for acute inflammation. Carrageenan is the phlogistic agent of choice for testing antiinflammatory

TABLE-3
ANALGESIC ACTIVITY OF THE AQUEOUS EXTRACT OF *Aporusa oblonga* ON TAIL FLICK RESPONSE IN RATS

Treatment	Dose (mg/kg, p.o.)	Pretreatment	30 min	60 min	90 min	120 min	150 min
Control	–	3.083±0.03333	4.233±0.04944	3.950±0.04282	4.350±0.04282*	4.317±0.04773	4.100±0.03651
Pethidine	5	3.067±0.03333	8.100±0.0365*1	8.383±0.03073*	8.283±0.03073*	8.150±0.04282*	7.400±0.03651*
MAO	100	3.233±0.03333**	4.083±0.03073**	5.983±0.03073*	6.150±0.04282*	6.000±0.03651*	6.483±0.03073*
MAO	200	3.267±0.04216*	5.333±0.03333*	5.567±0.04216*	5.567±0.03073*	7.433±0.03333*	7.950±0.04282*
MAO	400	3.300±0.03651*	5.600±0.03651*	7.583±0.03073*	8.367±0.03333*	9.100±0.03651*	7.817±0.03073*

Each value is the mean ± SEM of 5 rats. *$p < 0.01$ compared with control; Dunnett test. **$p < 0.05$ compared with control; Dunnett test.

TABLE-4
EFFECT OF *Aporusa oblonga* ON THERMIC STIMULUS-INDUCED PAIN IN RATS (HOT PLATE TEST)

Group	Dosage (mg/kg p.o)	Reaction time[a] (s)		
		30 min	60 min	90 min
Control	0	1.280 ± 0.08602	1.00 ± 0.05477	0.82 ± 0.07348
MAO	100	1.34 ± 0.05099	1.22 ± 0.05831	1.12 ± 0.03742*
MAO	200	1.46 ± 0.06782	1.34 ± 0.08124**	1.2 ± 0.09487**
MAO	400	1.5 ± 0.07071	1.36 ± 0.09274**	1.24 ± 0.1077**
Pethidine	5	1.7 ± 0.07071*	1.58 ± 0.03742**	1.46 ± 0.06782**

[a]Each value is the mean ± SEM of 5 rats. *$p < 0.05$, significant *compared to control. **$p < 0.01$, significant **compared to control; Dunnett test.

TABLE-5
ANTIINFLAMMATORY ACTIVITY OF A*porusa oblonga* ON CARRAGEENAN INDUCED RAT PAW OEDEMA IN THE RATS

Treatment and dose (mg/kg, p.o.)	Total increase in paw volume (mean ± SEM) (inhibition %)			
	1 h	2 h	3 h	4 h
Control	0.33 ± 0.007071	0.68 ± 0.01068	0.8540 ± 0.005099	0.97 ± 0.006782
Aspirin	0.116 ± 0.005099* (64.8)	0.168 ± 0.006633* (75.3)	0.23 ± 0.007483* (73.07)	0.31 ± 0.007348 (68.05)
MAO	0.238 ± 0.008602* (27.8)	0.29 ± 0.01122 (57.36)	0.61 ± 0.005477* (28.6)	0.74 ± 0.008124 (23.7)
MAO	0.206 ± 0.006782* (37.6)	0.23 ± 0.005099* (66.2)	0.45 ± 0.008531* (47.3)	0.53 ± 0.006782 (45.4)
MAO	0.16 ± 0.009274* (51.5)	0.20 ± 0.006633* (70.59)	0.35 ± 0.007071* (59.02)	0.42 ± 0.006782 (56.71)

Values are expressed as mean ± SEM (n = 5). Statistically significant from control group: *$p < 0.01$; Dunnett test.

TABLE-6
EFFECTS OF METHANOLIC EXTRACT OF *Aporusa oblonga* ON COTTON PELLET GRANULOMA IN RATS

Group	Dosage orally (mg/kg)	Increase in weight of pellets (mg)[a]	Inhibition (%)
Control	–	62.0 ± 0.7071	–
Indomethacin	5	20.0 ± 0.7071*	67.7
MAO	100	49.2 ± 0.8602*	20.6
MAO	200	33.4 ± 0.9274*	46.1
MAO	400	23.4 ± 0.6782*	62.2

Values are mean $p < 0.01$ ± SEM (n = 5); *$p < 0.01$ compared with control; Dunnett test.

drugs as it is not known to be antigenic and is devoid of apparent systemic effect[24]. Moreover, the model exhibits a high degree of reproducibility[25]. The probable mechanism of action of carrageenan-induced inflammation is biphasic; the first phase is attributed to the release of histamine, serotonin and kinins in the first h; while the second phase is attributed to the release of prostaglandins and lysosome enzymes in the second to the 3 h[26]. The ability of the extract to inhibit carrageenan-induced paw oedema suggests it possesses a significant effect against acute inflammation. The extract caused a significant hypothermal activity against yeast-induced pyrexia in rabbits. Subcutaneous injection of yeast induces pyrexia by increasing synthesis of prostaglandin and is used to screen agents for antipyretic effect[27]. The Antinociceptive and antipyretic activities may be attributed to the presence of phenols, polyphenols, saponins and steroids found in crude extract. Further studies may reveal the exact mechanism of action responsible for the analgesic and antiinflammatory activities of *Aporusa oblonga*.

Conclusion

The present findings support the hypothesis that *A. oblonga* has antiinflammatory potential against some phlogistic agents, immunomodulatory effect and also has antinociceptive action probably mediated *via* opioid receptors. Moreover *Aporusa oblonga* stem bark powder may prove to be an important indigenous drug in future for the treatment of pyrexia. Further identification of the triggers of inflammation and unravelling the details of inflammatory pathways may eventually furnish new therapeutic targets for the treatment of inflammation and other related disorders.

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TABLE-7
ANTIPYRETIC ACTIVITY OF *Aporusa oblonga* ON BREWER'S YEAST-INDUCED PYREXIA IN EXPERIMENTAL RABBITS

Treatment	Dose (mg/kg)	Average rectal temperature (ºC)				
		Pretreatment	After treatment			
		0	1 h	2 h	3 h	4 h
Control	5 mL/kg	39.3 ± 0.1327	41.6 ± 0.4889	41.6 ± 0.4889	41.6 ± 0.4913	41.52 ± 0.4598
Paracetamol	200	39.5 ± 0.2694	40.66 ± 0.6329	40.62 ± 0.5774	40.3 ± 0.5860	39.1 ± 0.09274*
MAO	100	39.7 ± 0.1871	41.0 ± 0.4087	41.0 ± 0.3886	40.2 ± 0.5229	39.7 ± 0.2112*
MAO	200	36.9 ± 0.09274*	37.8 ± 0.08602*	37.7 ± 0.09274*	37.4 ± 0.07483*	37.0 ± 0.07071*
MAO	400	39.5 ± 0.2441	40.4 ± 0.5113	40.2 ± 0.5335	40.1 ± 0.4354	39.7 ± 0.2015*

Values are mean $p < 0.01$ ± SEM (n = 5) *$p < 0.01$ compared with control; Dunnett test.

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