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Analgesic and Anti-inflammatory Activities of the Fruit Extract of *Ampelocissus latifolia (Roxb)* on Laboratory Animals

B. K. Das[1*], U. K. Fatema[2], M. S. Hossain[2], R. Rahman[2], M. A. Akbar[2] and F. Uddin[2]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh.
[2]Department of Pharmacy, North South University, Bashundhara, Dhaka-1229, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Author BKD designed the study, wrote the protocol and checked the manuscript. Authors UKF and MSH conducted the experimental works and wrote the first draft of the manuscript. Authors RR and KF helped to finish the experimental works. Author FU performed the statistical analysis. Author BKD managed the literature searches and analyses of the data. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim of Study: This study was aimed to evaluate the possible analgesic and anti-inflammatory properties of the ethanol extract of fruit of *Ampelocissus latifolia* (Roxb).
Study Design: Assessment of analgesic and anti-inflammatory activities.
Place and Duration of Study: Department of Pharmacy, North South University, Dhaka, Bangladesh, between June 2012 and March 2013.
Methodology: The crude ethanol extract was investigated for the anti-inflammatory effect on Long Evans rats using carrageenan induced rat paw edema method. For anti-inflammatory study, 20 rats were divided into 4 different groups, each receiving either distilled water, standard drug or the extract at the doses of 250 and 500mg/kg body weight (BW). The analgesic activity was evaluated by hot plate and acetic acid induced writhing method in Swiss albino mice divided into 4 different groups (control, standard and extract at two different doses of 250 and 500 mg/kg BW).

**Corresponding author: Email: bkdas72@du.ac.bd, bkdas72@yahoo.com;*

Results: The results of preliminary phytochemical study revealed the presence of alkaloids, flavonoids, tannins, glycosides in significant amounts. The results of anti inflammatory activity study showed that the fruit extracts at a dose of 250 mg/kg and 500 mg/kg inhibited carrageenan-induced paw edema in Long- Evans rats. Both the extracts were able to show a dose dependent anti-inflammatory activity as compared to diclofenac sodium used as a standard drug. The extract elicited a highly significant ($p<0.001$) analgesic activity in a dose dependent manner on hot plate method, acetic acid-induced writhing test. In the hot plate method, the extract increased the reaction time of heat sensation to 94.71% and 82.7% at the doses of 250 and 500 mg/kg BW while that of the standard drug was 61.45% at the 3rd hour of study. In acetic acid induced writhing test, the percent inhibition of writhing response by the extract was 50.57% and 59.77% at 250 and 500 mg/kg doses respectively ($p<0.001$).
Conclusion: The anti-inflammatory and analgesic effect of the ethanol fruit extract of *Ampelocissus latifolia* (Roxb) may be due to the presence of various chemical constituents especially flavonoids, tannins, alkaloids or terpenoids. These experimental findings would further establish the scientific basis of the traditional uses of the plant in the management of inflammatory conditions as well as control of pain.

Keywords: Ampelocissus latifolia; anti-inflammatory; carrageenan; analgesic.

1. INTRODUCTION

Herbal medicine has been widely practiced from ancient period to the present day with much more attention than allopathic drugs because of their minimum side effects and cost-effectiveness throughout the world. Herbal drugs obtained from plants are believed to be much safer in the treatment of various diseases [1]. Traditional medicine; especially the folk herbal medicines have recently been receiving heightened interests worldwide. Such an old health care system had been developed in different corners of the world where they were living in close interaction with the nature. Information from ethnic groups on indigenous traditional herbal medicines had always played a vital role in the discovery of novel chemotherapeutic agents form plants [2].

Inflammation is the part of biological reaction of vascular tissues to external harmful stimuli, such as pathogens, damaged cells, or irritants [3]. Inflammation is not like infection, even though inflammation may be caused by infection itself. The difference between two is that infection is caused by the attack of microorganism whereas inflammation is a reaction of organism to pathogens [4]. Drugs that are currently used for the management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. All these drugs have some potential side effects amongst which the gastrointestinal ailments are the commonest [5]. As the result of the inherent problems associated with the current non-steroidal as well as steroidal anti-inflammatory agents, there is continuous search for alternative drugs especially from natural sources [6]. Large numbers of herbal extracts as well as products being employed in the treatment of painful inflammatory disorders.

Ampelocissus latifolia (syn. Vitis latifolia) is a widely used medicinal plant in Vitaceae family (Grape family) native to the India subcontinent (Bangladesh, India, Nepal, and Pakistan). They are slender climber. Fruits are non edible wild grape usually spherical and black normally 2 seeded and rarely 3 seeded [7]. The common name of the plant in Bengali is

"Jungli angoor" and it is commonly known as "Panibel" in India. It has been used as an herbal medicine in dyspepsia, gout, indigestion, tuberculosis, and as an antidote [8]. Fresh crushed tuber of *A. latifolia* is boiled in castor oil and then applied externally for the treatment of gout. Crushed tuber is given to animal to cure fractured bone. Infusion of whole plant is used as tonic by old age person. Mixture of *Urginea indica* and *A. latifolia* are used for diseases related to respiratory tract like cough, cold, asthma etc [9]. *A. latifolia* roots have been used for the treatment of snake bite and for its astringent effect. The decoction of the root is also used in chronic dysentery. The sandals of Bihar used this plant for muscular pains, sores and fractured bones [10]. The whole plant extract of organic solvents is found to have significant anti-inflammatory activity. The extracts were also studied for acute toxicity and were found to be safe up to a maximum dose of 500mg/kg. Al these extracts were studied for preliminary phytochemical investigation [11].

Although numerous studies have shown the medicinal values of this plant, its anti-inflammatory and analgesic effects of fruit extract are not yet reported. As a part of our continuing study on chemical and biological investigation of different plants, the present study was attempted for the first time to investigate the anti-inflammatory and analgesic activity of *A. latifolia* to search for newer, safer and more potent anti-inflammatory as well as analgesic agent and we herein delineate the results of our study.

2. MATERIALS AND METHODS

2.1 Collection and Identification of the Plant Material

The mature fruits of *A. latifolia* were collected in July 2012 from Savar, Dhaka, Bangladesh. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (accession no. - DACB 37898)

2.2 Preparation of Extracts

The fruits were first shade dried for a week. Then the crushed fruits were ground into coarse powder with the help of a mechanical grinder. The whole powder (approximately 500gm) was extracted by percolation with 95% ethanol and kept for a period of 3 days accompanying occasional shaking and stirring. The whole mixture was then undergone a coarse filtration by a piece of clean, white cotton material followed by a second filtration through whatman filter paper. The filtrate (ethanol extract) obtained was evaporated by Rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and at 68ºC temperature. It rendered a gummy concentrate of dark brown color. This gummy concentrate was designated as ethanol extract of *A. latifolia* (EEAL). Then the crude extract was dried by freeze drier and preserved at 4ºC.

2.3 Experimental Animals

Young Swiss albino mice aged about 4-5 weeks with average weight of 25-35 gm and adult Long-Evans Rats of either sex having average weight of 100-130gm were used for the experiment and maintained in the animal house of the Department of Pharmacy, North South University for acclimation. The animals were originally obtained from International Centre for Diarrheal Disease Research, Bangladesh (*ICDDR*, *B*). They were housed in standard cages under standard environmental conditions of room temperature at 24±1ºC and 55-65% relative humidity with 12 hour dark light cycle and provided with standard diet

for rodents and water *ad libitum*. All experiments involving animals were conducted according to the UK Home Office regulations (UK Animals Scientific Procedures Act 1986) and the 'Principles of Laboratory Animal Care' (National Institutes of Health publication no. 86-23, revised 1985). The rat and mice had no access to food during the whole day of experiment. The animals were also not provided with water during the experiment as the vehicle for administering samples was water and the animals of control group were given only the vehicle.

2.4 Method for Phytochemical Analysis

The freshly prepared crude extract was qualitatively tested for the identification of chemical constituents, such as, alkaloids, flavonoids, steroids, glycosides, saponins, terpenoids, gums and tannins. The tests were carried out by the method described by Harborne and Sazada et al. [12] and in each test 10% (w/v) solution of the extract was taken unless otherwise mentioned in individual test.

2.5 Analgesic Studies

2.5.1 Hot plate test

The hot plate test method was employed to assess the analgesic activity in accordance with the method described previously with minor modification [13]. The experimental animals were divided into control, positive control and test groups with six mice in each group. The animals of test groups received test samples at the doses of 250 and 500mg/kg body weight, positive control group was administered diclofenac sodium at the dose of 10mg/kg body weight and vehicle control group was treated with 1% Tween 80 solution in distilled water at the dose of 10 ml/kg body weight orally. In this test, the animals were positioned on Eddy's hot plate kept at a temperature of 55±0.5ºC. The test samples and the standard drug were administered 30 minutes before the beginning of the experiment. Mice were observed before and at 30, 60, 120, 180 and 240 min after administration. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60, 120,180 and 240 min after oral administration of the samples. A cut-off period of 20 seconds was observed to avoid the damage of the paw. The anti-nociceptive response latency was recorded from the time between placement and licking of fore or hind paws or jumping movements of the animals. Percent analgesic score was calculated as, $PAS=T_a-T_b/T_a \times 100$.

Where, T_b = Reaction time (in second) before drug administration; T_a = Reaction time (in second) after drug administration.

2.5.2 Acetic acid induced writhing method

To evaluate the analgesic effects of the plant extract, the method described by Dharmasiri JR et al. [14] was used with slight modifications [14]. Different groups of six mice each received orally normal saline solution (10ml/kg) (i.e. control), diclofenac sodium (10mg/kg), or plant extract (250 and 500mg/kg). Thirty minutes later, 0.6% acetic acid (10ml/kg) solution was injected intraperitoneally (i.p.) to all the animals in the different groups. The number of writhes (abdominal constrictions) occurring between 5 to 15 min after acetic acid injection was counted. A significant reduction of writhes in tested animals compared to those in the control group was considered as an analgesic response.

The percentage inhibition of writhing was calculated using the following formula:

$$\text{Percent Inhibition} = (1\text{-}W_t/\ W_c) \times 100$$

Where, W_c and W_t represent the average number of writhing produced by the control and the test group, respectively.

2.6 Method for the Evaluation of Anti-inflammatory Effect

The anti-inflammatory activity of the ethanol extract of *A. latifolia* was investigated on carrageenan induced inflammation in rat paw following an established method [15]. Rats were randomly divided into four groups, each consisting of five animals, of which group I was kept as control giving only distilled water. Group II was standard which received diclofenac sodium (10mg/kg) as the reference standard for comparison while Group III and Group IV were given the test material at a dose of 250 and 500mg/kg body weight respectively. Half an hour after the oral administration of the test materials, 1% carrageenan was injected to the right hind paw of each animal [16]. The volume of paw edema was measured at 0, 1, 2, 3, and 6 hours using Plethysmometer (Model 7141, UGO Basile, Italy) after administration of carrageenan. The left hind paw served as a reference non-inflamed paw for comparison.

The average percent increase in paw volume with time was calculated and compared against the control group. Percent inhibition was calculated using the formula-

$$Percent\ Inhibition = \frac{Mean\ paw\ volume\ of\ control - Mean\ paw\ volume\ of\ test}{Mean\ paw\ volume\ of\ test} \times 100$$

2.7 Statistical Analysis

The data are expressed as the mean ± SEM analyzed by one-way analysis of variance (ANOVA) and Dunnett's *t*-test was used as the test of significance. P value<0.05 was considered as the minimum level of significance. All statistical tests were carried out using SPSS statistical software.

3. RESULT AND DISCUSSION

3.1 Phytochemical Analysis

Preliminary phytochemical screening of the ethanol extract of *A. latifolia* fruit revealed the presence of various bioactive components of which flavonoids, alkaloids, terpenoids, tannins, gums and carbohydrates were the most prominent and the result of phytochemical test has been summarized in the Table 1.

Table 1. Phytochemical analysis of the EEAL fruit

Extract	Tannin	Flavonoid	Saponin	Gum	Alkaloid	Terpenoid
EEAL	++	+++	++	++	+++	+++

Symbols '+++' indicates presence in high concentration; '++' indicates presence in moderate concentration

3.2 Analgesic Activity

3.2.1 Hot plate method

Results of hot plate test are presented in Table 2 for the crude extract of *A. latifolia.* The fruit extract of the plant significantly increased the reaction time of heat sensation in mice at the doses of 250 and 500mg/kg BW and the percentage protection is almost equivalent to the respective doses. In the 3rd hour of study, the extract increased the reaction time of heat sensation to 94.71% and 82.7% at the doses of 250 and 500mg/kg BW respectively while that of the standard drug was 61.45% and the results were found to be highly statistically significant (P<0.001). The extract exhibited a dose dependent increase in latency time when compared with control.

Table 2. Effect of the EEAL on latency to hot plate test

Group	Reaction time at different time intervals (in sec)					
	0 Hour	½ Hour	1 Hour	2 Hours	3 Hours	4 Hours
Control	8.30±0.61	7.42±0.80	7.50±0.73	7.50±0.64	7.19±0.04	6.78±0.60
Standard	7.42±0.88	9.52±1.22 (28.3)	10.98±1.33 (48.58)	11.98±1.07*** (59.2)	13.38±0.85*** (61.45)	10.54±1.43 (42.04)
EEAL 250mg/kg	7.60±0.50	9.56±1.42 (25.78)	11.44 ±1.96 (50.52)	13.52±0.92** (77.69)	14.80±1.31*** (94.71)	12.78±1.78 ** (68.15)
EEAL 500mg/kg	7.70±1.70	8.96±1.34 (21.08)	10.36±0.95 (40)	12.22±1.46*** (65.13)	13.52±1.02*** (82.7)	13.36±1.38 (80.54)

*Data are represented as the mean ± SEM, (n=5); Values in parentheses indicate percent increase in reaction time; *P<0.05, **P<0.01, ***P<0.001 were considered statistically significant as compared to control*

3.2.2 Acetic acid-induced writhing test

Inhibition of licking response in mice due to the administration of the test drugs during acetic acid-induced writhing test is shown in Table 3. The oral administration of both doses of *A. latifolia* fruit extract significantly (p<0.001) attenuated the acetic acid-induced abdominal writhes in mice in a dose dependent fashion. The percent inhibition of writhing response by the extract was 51.78% and 60.9% at 250 and 500mg/kg doses respectively while the standard diclofenac sodium (10mg/kg) showed 67.81% inhibition in comparison with the control.

Table 3. Effect of the EEAL on acetic acid-induced writhing in mice

Groups	Dose	Route	No. of writhing	% Inhibition
Control	10ml/kg	p.o	17.4±2.50	-
Standard	10mg/kg	p.o	5.60±1.66***	67.81
EEAL	250mg/kg	p.o	8.40±1.07***	51.78
EEAL	500mg/kg	p.o	6.80± 0.37***	60.9

*Data are represented as the mean ± SEM, (n=5); ***P<0.001 was considered statistically significant as compared to control*

3.3 Anti-Inflammatory Result

On the basis of experimental data, it was observed that there was significant and dose dependent anti-inflammatory activity of ethanol fruit extract and the data is shown in Table 4. The doses are administered orally at the dose of 250mg/kg and 500mg/kg of the body weight of the animals. After 2 hours diclofenac sodium produced 67.75%, ethanol extract 71.55% and 76.45% inhibition at a dose of 250 and 500mg/kg, respectively. At the end of 3 hours, ethanol extract shows the inhibition 45.45% and 47.4% at a dose of 250mg/kg and 500mg/kg inhibition respectively as compared to diclofenac sodium (43.78%).

Table 4. Anti-inflammatory activity of EEAL using carragseenan - induced rat paw edema method

Treatment group & dose	Paw volume in ml				
	0 Hour	1 Hour	2 Hours	3 Hours	6 Hours
Control 10ml/kg	0.62±0.04	0.85±0.04	1.02 ±0.06	1.40±0.08	1.21±0.07
Standard 10mg/kg	0.67±0.03	0.93±0.04* (38.46)	1.13±0.06* (67.75)	1.24±0.09** (84.02)	0.97±0.04* (43.78)
EEAL 250mg/kg	0.68±0.04	0.94±0.06 (39)	1.01±0.01 (71.55)	1.20±0.13* (76.24)	0.99±0.04 (45.45)
EEAL 500mg/kg	0.65± 0.02	0.90± 0.03 (37)	1.15±0.01* (76.45)	1.22±0.03** (87.15)	0.96±0.02* (47.4)

Data are represented as the mean ± SEM, (n=5); Values in parentheses indicate percent inhibition of paw edema; p<0.05, ** p<0.01, were considered statistically significant as compared to control.*

Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception [17]. The ability of the extract to prolong the reaction latency to pain thermally-induced in mice by the hot plate further suggests central analgesic activity. The acetic acid induced abdominal constriction method is widely used for the evaluation of peripheral anti-nociceptive activity [18]. It is very sensitive and able to detect anti-nociceptive effects of compounds at dose levels that may appear inactive in other methods like the tail-flick test [19]. Local peritoneal receptors are postulated to be partly involved in the abdominal constriction response [20]. The method has been associated with prostanoids in general, e.g. increased levels of PGE2 and PGF2α in peritoneal fluids [21], as well as lipoxygenase products by some researchers [22]. Therefore the results of the acetic acid induced writhing strongly suggest that the mechanism of action of this extract may be linked partly to lipoxygenases and/or cyclo-oxygenases pathways. The extract at the doses tested was shown to possess anti-nociceptive activity evident in all the nociceptive models, signifying it possesses both central and peripherally mediated activities.

Carrageenan is the phlogistic agent of choice for testing anti- inflammatory drugs as it is more antigenic and is devoid of apparent systemic effect [23]. Carrageenan model of inflammation is said to be biphasic, with the first phase attributed to the release of histamine, serotonin and kinins in the first hour; while the second phase is attributed to the release of prostaglandins and lysosome enzymes in the second to the third hour [24]. The extract inhibited both the first and second phases of inflammation. The ability of the extract to inhibit carrageenan induced paw edema suggests that it possesses a significant effect against acute inflammation. The extract (250mg/kg) also caused marked inhibition of carrageenan-induced hind paw edema in rats as compared with diclofenac sodium (10mg/kg), the standard anti-inflammatory agent used.

The phytochemical screening of the ethanol fruit extract of *A. latifolia,* revealed the presence of alkaloids, carbohydrates, flavonoids, saponins and tannins. Flavonoids, saponins and tannins have been shown to exert analgesic effect on acetic acid induced writhing test [25]. The flavonoids, saponins and tannins might be responsible in part for the observed analgesic and anti-inflammatory effect.

4. CONCLUSION

On the basis of our findings, it can be inferred that the ethanol fruit extract of *A. latifolia* has analgesic and anti-inflammatory activities. These activities are dose-related. However, further study is needed in order to understand the precise mechanism. Studies of pure active compounds of the extract must be conducted for further pharmacological and toxicological characterization.

CONSENT

Not applicable.

ETHICAL APPROVAL

The authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have been performed in accordance with the ethical standards.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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