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Anti-inflammatory and Analgesic Activities of *Alpinia nigra* Fruit Extract in Laboratory Animals

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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 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: This study was aimed to evaluate the possible anti-inflammatory and analgesic properties of the ethanol extract of fruit of *Alpinia nigra* (Zingiberaceae).
Study Design: Assessment of anti-inflammatory and analgesic activity.
Place and Duration of Study: Department of Pharmacy, North South University, Dhaka, Bangladesh, between June 2012 and February 2013.
Methodology: The crude extract was investigated for anti-inflammatory effect on Long Evans rats using carrageenan induced 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 500 mg/kg body weight. The analgesic activity was evaluated by hot plate; acetic acid induced writhing method in Swiss Albino mice divided into 4 different groups (control, standard diclofenac sodium and extract at two different doses of 250 and 500 mg/kg body weight).
Results: The results of preliminary phytochemical analysis revealed the presence of

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alkaloids, flavonoids, tannins, glycosides in significant amounts. The present study assessed anti-inflammatory activity of its fruit extracts at a dose of 250 mg/kg and 500 mg/kg against 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. The extract elicited a highly significant ($p<0.001$) analgesic activity in a dose dependent manner on hot plate and acetic acid induced writhing methods.

Conclusion: The anti-inflammatory and analgesic effect of the ethanol fruit extract of *A. nigra* 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: Alpinia nigra; Anti-inflammatory; Analgesic; Carrageenan; Paw Edema; Ethanol Extract.

1. INTRODUCTION

The plant kingdom represents an enormous reservoir of pharmacologically valuable molecules to be discovered [1]. Among the estimated 350,000 plant species on the earth, only a small percentage has been pharmacologically investigated and the fraction submitted to biological or pharmacological screening is even smaller. Over the last decade, we have witnessed a substantial acceleration of the changes in the drug discovery process as a whole and these changes have necessarily had a substantial impact in the area of natural products. Compounds of natural origin play a major role as “drugs” and as “lead structure” for the development of synthetic molecules [2]. Natural products are being widely used in the form of medicinal plants as “path finder” molecules for the discovery and validation of drug targets, herbal extracts and finished products or phytopharmaceuticals [3].

Inflammation is the part of biological reaction of vascular tissues to external harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is not like infection, even though the 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. Drugs that are currently used for the management of inflammatory conditions are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. All these drugs carry potential toxic effects, of which gastrointestinal side effects are the commonest. As the result of the inherent problems associated with the current non-steroidal as well as steroidal anti-inflammatory agents, there is a continuous search for alternative agents especially from natural sources. Large numbers of herbal extracts as well as products are currently being employed in the treatment of painful inflammatory disorders [4].

Due to having adverse side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking those effects are being searched all over the world as alternatives to NSAIDs and opiates. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80% of the world population rely mainly on plant-based drugs [5].

Alpinia nigra (Bengali name: Jangli Ada, Family: Zinziberaceae) is extensively grown in Bangladesh, India and Srilanka [6]. Previous phytochemical investigation revealed two flavone glycosides, astragalin and kaempferol-3–*O*-glucuronide along with three dihydroxypropyl esters from the seed clusters of this plant [7]. Many pharmacological studies have reported that *Alpinia nigra* possess several biological activities including, antioxidant, antiprotozoal, hepatoprotective and anti-inflammatory effects [8,9]. Besides significant studies regarding ethnomedical uses of Zingiberaceous plants as well as the plants of *Alpinia* genus have been reported [10,11].

Although numerous studies have shown the medicinal values of this plant, its anti-inflammatory and analgesic effects on fruit part 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. nigra* 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. nigra* were collected from Savar, Dhaka, Bangladesh. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka. The accession no is DACB 37947.

2.2 Phytochemical Evaluation

2.2.1 Preparation of extracts

The fruits were first sun dried for a week. Then the crushed fruits were ground into coarse powder with the help of a mechanical grinder. The whole powder (about 500 gm) was extracted by cold extraction with 95% ethanol and kept for 72 hours with occasional stirring and shaking. The crude extract was then filtered off and the filtrate obtained was evaporated to dryness *in vacuo* by Rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) to get a concentrated gummy mass of dark brown color. This gummy concentrate was designated as ethanol extract of *Alpinia nigra* (EEAN) The crude extract was finally dried by freeze drier and preserved at 4ºC.

2.2.2 Phytochemical tests

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.

2.3 Experimental Animals

Swiss-albino mice (age 4-5 weeks, average weight 25-35 gm) and adult Long-Evans rats of either sex (average weight 100-130 gm) were used for these study. 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 food 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 influence of circadian rhythms was avoided by starting all experiments at 8.30 a.m.

2.4 Method for the Evaluation of Anti-inflammatory Effect

The anti-inflammatory activity of the ethanol extract of *Alpinia nigra* was investigated on carrageenan induced inflammation in rat paw following an established method [13]. 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 (10 mg/kg) as the reference standard for comparison while Group III and Group IV were given the test material at a dose of 250 and 500 mg/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 [14]. The anti-inflammatory activity was assessed by measuring the volume of paw edema at 0, 1, 2, 3, and 6 hours after the administration of carrgeenan using Plethysmometer (Model 7141, UGO Basile, Italy). The left hind paw was non-inflamed and was used as a reference for comparison.

After finding the mean paw volume of test and control groups, percent inhibition of paw edema was calculated using the following formula-

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

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 [15]. 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 500 mg/kg body weight, positive control group was administered diclofenac sodium at the dose of 10 mg/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 antinociceptive 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,

$$PAS = T_a\text{-}T_b/T_a \times 100$$

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. [16] was used with slight modifications. Different groups of six mice each received orally normal saline solution (10 ml/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 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 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. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

Preliminary phytochemical screening of the ethanol extract of *Alpinia nigra* 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 EEAN fruit

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

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

3.2 Anti-inflammatory Result

On the basis of experimental data, it was observed that there was significant and dose dependent anti-inflammatory activity of ethanolic fruits extract. The extracts were

administered orally at the doses of 250 mg/kg and 500 mg/kg of the body weight of the animals. After 2 hour diclofenac sodium produced 67.75%, ethanolic extract 88.48%, 80.04% inhibition at a dose of 250, 500 mg/kg, respectively. At the end of 3 hour ethanolic extract shows the inhibition 76.22% and 60.82% at a dose of 250 mg/kg and 500 mg/kg inhibition respectively as compaired to diclofenac sodium (standard) 92.30% (Table 2).

Table 2. Anti inflammatory activity study of EEAN by carrageenan induced paw edema method

Treatment group & dose	Paw volume in ml				
	0 Hour	1 Hour	2 Hours	3 Hours	6 Hours
Control 10 ml/kg	0.62±0.04	0.85 ± 0.04	1.02 ±0.06	1.44±0.08	1.34±0.07
Standard 10 mg/kg	0.67± 0.03	0.93 ± 0.04* (38.46)	1.13±0.06* (67.75)	1.38±0.09** (92.30)	0.87±0.04* (28.99)
EEAN *250* mg/kg	0.81±0.04	1.24±0.06 (52.94)	1.53±0.01 (88.48)	1.43±0.13* (76.22)	0.97±0.04 (19.11)
EEAN 500 mg/kg	0.82± 0.02	1.27± 0.03 (54.50)	1.48±0.01* (80.04)	1.32±0.03** (60.82)	0.99± 0.02* (20.68)

*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.*

3.3 Analgesic Activity

3.3.1 Hot plate method

Results of hot plate test are presented in Table 3 for the crude extract of *Alpinia nigra.* The bark extract of the plant significantly increased the reaction time of heat sensation in mice at the doses of 250 and 500 mg/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 73.39% and 78.62 % at the doses of 250 and 500 mg/kg BW respectively while that of the standard drug was 59.2% 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 3. Effect of EEAN on latency in 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±0.40	6.78±0.60
Standard	7.42±0.88	9.52±1.22 (28.3)	10.58±1.33 (36.1)	11.98±1.07*** (48.58)	13.38±0.85*** (59.2)	10.54±1.43 (62.61)
EEAN 250 mg/kg	8.12±0.50	9.56±1.42 (25.78)	10.84±1.95 (48.3)	12.44±0.92** (53.2)	14.08±1.31*** (73.39)	12.08±1.78** (48.76)
EEAN 500 mg/kg	7.65±1.70	8.58±1.34 (21.08)	9.32±0.95 (33.45)	12.1±1.46*** (58.16)	13.68±1.02*** (78.62)	12.44±1.38 (61.45)

*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.*

3.3.2 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 4. The oral administration of both doses of *Alpinia nigra* 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 52.67% and 62% at 250 and 500 mg/kg doses respectively while the standard diclofenac sodium (10 mg/kg) showed 67.81% inhibition in comparison with the control.

Table 4. Effect of EEAN on acetic acid-induced writhing in mice

Group	Dose	Route	No. of writhing	% Inhibition
Control	10 ml/kg	p.o	17.4±2.50	-
Standard	10 mg/kg	p.o	5.60±1.66***	67.81
EEAN	250 mg/kg	p.o	8.20±0.58***	52.67
	500 mg/kg	p.o	6.60± 0.67***	62

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

Inflammation is caused by release of chemicals from tissues and migrating cells. Most strongly implicated are the prostaglandins (PGs), leukotrienes (LT5), histamine, bradykinin, and, more recently, platelet activating factor (PAF) and interleukin-1. The healing process of inflammation is biphasic, namely, primary and secondary phases. Histamine is involved in the primary phase whereas prostaglandins are associated with the secondary phase. Both are known as inflammatory mediators. The extract exhibited consistent anti-inflammatory activity irrespective of the model used, and this suggests that anti-inflammatory effect may be due to the inhibition of prostaglandin synthesis [17].

Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception. 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].

Acetic acid-induced writhing is a well recommended protocol for evaluating medicinal agents for analgesic property. The pain in this model is induced by the liberation of endogenous substances as well as some other pain mediators such as arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis [19]. This pain paradigm is widely used for the assessment of peripheral analgesic activity due to its sensitivity and response to compounds at a dose that is not effective in other models.

Our findings strongly suggest that peripheral analgesic activity and its mechanisms of action may be mediated through inhibition of local peritoneal receptors which may involve cyclooxygenase inhibition. The profound analgesic activity of this extract may be due to interference of its active principle(s) with release of pain mediators. Preliminary phytochemical analysis of the extract revealed the presence of tannins, flavonoids, saponins, alkaloids in moderate to high amounts. These phytochemicals are reported to be responsible for analgesic and anti-inflammatory activities [20,21,22]. Therefore the observed anti-

inflammatory and analgesic activities of the fruit extract of A. nigra is assumed to be due to the presence of these chemical substances.

4. CONCLUSION

Findings from this study demonstrated that *Alpinia nigra* possesses analgesic (peripheral and centrally acting) and anti-inflammatory activities providing justification for folkloric use of the plant. 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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