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ANALGESIC AND ANTI-INFLAMMATORY STUDIES OF METHANOL EXTRACT OF *I. FRUTESCENS* ON EXPERIMENTAL MICE

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ABSTRACT

Almost all analgesic and anti-inflammatory drugs cause hyperacidity and peptic ulcer. To find the new source of drug, present study was carried out to evaluate analgesic and anti-inflammatory effects of methanol extract of *I. frutescens*. *I. frutescens* showed significant analgesic effects ($p<0.001$) on acetic acid induced writhing of mice compare to control group where diclofenac sodium was used as a reference standard. *I. frutescens decreased* the carrageenan induced paw edema notably ($^{*}p<0.05$, $^{**}p<0.01$ and ***P-value<0.001) compare to control group in which standard was diclofenac sodium. phytochemical screening indicated the presence of Glycoside, Flavonoid, Saponin and Steroid. Analgesic and anti-inflammatory effects may be due to the presence of Glycoside, Flavonoid but further study needed.

KEYWORDS: *Analgesic, anti-inflammatory, acetic acid, carrageenan, I. frutescens.*

INTRODUCTION

Plants are most important and essential component of the universe. From the beginning of time plants have been used as medicine by human being. Plants were identified as a important source of medicine for treatment through various observations and experiments at the early stages of human civilization.[1] The use of these medicinal plants is increasing day by day in many countries where natural product contribute 35% of total drugs. At present, various parts of plant and thousands of plant primary and secondary metabolites are being successfully used for the management of variety of diseases.[2] In Bangladesh thousands of species are present having medicinal value and different parts of several medicinal plants are used to cure specific ailments since ancient times. Combinations of secondary product present in plant such as alkaloids, steroids, tannins, phenol compounds, resins, gums, flavonoids and fatty acids are capable of producing definite beneficiary physiological action on body.[3] Now a day's cerebral and coronary artery diseases are the alarming causes of death around the world. According to many recent researchers it has demonstrated that abnormal inflammatory cells form a plaque and play an essential role in the pathogenesis and progression of atherosclerosis.[4]Anti-inflammatory agents have significant effects on the prevention and treatment of atherosclerosis and coronary artery diseases.[5] Analgesic drugs act on the peripheral and central nervous systems on the other hand Narcotic drugs, such as morphine shows analgesic activity through inhibiting the delivery of pain impulses.[6-7] Between Peripheral drugs include paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs) only NSAIDs possess analgesic and anti-inflammation activity because it inhibit cyclooxygenases (COXs) which decrease prostaglandin (PG) synthesis, that reduces pain and inflammation consequently. Because of some common side effects, such as gastrointestinal (GI) hemorrhage, clinically use of NSAIDs is limited.[8] Acetaminophen is a popular and effective pain-reliever because it can relieve both mild to moderate pain and relatively inexpensive[9] As a result It is used in combination with other active ingredients in many cold, sinus, and cough medications because of these multiple uses people must be considered regarding the cumulative effect of acetaminophen who talking multiple drugs containing acetaminophen.[10-14] *Anti-inflammatory* is the characteristic of a component or treatment that reduces or relives inflammation or swelling. As for instance Non-steroidal anti-inflammatory drugs (NSAIDs) aspirin, ibuprofen, and naproxen, reduce pain by counteracting the cyclooxygenase (COX) enzyme is responsible for prostaglandins synthesis which create inflammation.[15] Prescription as well as over-the-counter NSAIDs except aspirin increases the risk of myocardial infarction and stroke.[16] According to two studies in 2012 and 2013 it has been found that regular use of aspirin for over ten years increase the risk of macular degeneration.[17-18]

Ichnocarpus frutescens of the dogbane family is a species of flowering plant is known as the English common name black creeper and is native to China, India, Southeast Asia, and northern Australia.[19-20] It is a woody shrub with lianas sprawling to 10 meters in maximum length and 6 centimeters in diameter. The bark produces a creamy white sap. The inflorescence is a head of several flowers and the fruit is a follicle.[23] The plant traditionally uses in controlling rheumatism, asthma, cholera, and fever.[21] Some studies have suggested that extracts obtained from this plant inhibit tumors,[24] protect liver cells from damage in acetaminophen overdose.[22]

METERIALS AND METHODS

Plant Materials

The flowering plant of *Ichnocarpus frutescens* were collected from Tangail district, Bangladesh .

Drying and Grinding

The collected plants were separated from undesirable materials or plants or plant parts. They were dried in the sun for one week after cutting into small pieces. The plant parts were ground into coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Preparation of Plant Extract

About 300 gm of powdered sample was taken in a clean, flat-bottomed glass container and soaked in 1500 ml of 90% methanol. The container with its contents was sealed and kept for a period of 10 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by apiece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate was kept in an open space to evaporate the solvent thus crude extract was obtained. Fine powders of the flowering plant of *I. frutescens* are dissolved in 90% methanol and then evaporation the solvent.

Phytochemical Screening[25-27]

Phytochemical studied of methanolic extract of plant material extract was carried out for preliminary chemical investigation for the direction of practical pharmacognosy text book.

Drugs and chemicals

Carrageenan was purchased from Otto chemicals, India. The standard drug Diclofenac-Na was purchased from Square Pharmaceuticals Limited of Bangladesh. Acetic acid, methanol and other chemicals supplied from laboratory of Bangladesh University were analytical grade.

Experimental animals: Eight week-old Swice albino mice (27-30g) purchased from Jahangirnagar University, Dhaka, Bangladesh and were housed in animals cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 hr light: 12 hr dark cycle). The mice were feed with standard pellet diet taken from, Jahangirnagar University Dhaka. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute.

Analgesic activity

Acetic acid induced writhing method: For analgesic test all mice were divided into four groups. Each group comprises 4 mice. Control group (received 0.5% methyl cellulose, per oral), Standard Group (received Diclofenac 10mg/kg intraperitoneally), and *I. frutescens* extract Group (received 300mg/kg *I. frutescens* extract per oral). The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30mins before intraperitoneal administration 10ml/kg of .7% acetic acid but Diclofenac-Na was administered intraperitoneally 15 minutes before the acetic acid injection, the mice were observed for specific contraction of body referred to as "writhing" for the next 10minutes.[28] Percentage protection of acetic acid induced writhing was calculated by the formula.

Percentage protection = (Wc-Wt)/Wc x100.

Where, wc is the mean values of control group and Wt is the mean values of treated group.

Anti-inflammatory activity

Inflammation (paw edema) was induced by injecting 0.1ml of 1% Carrageenan in physiological saline into the subplantar tissues of the left hind paw of each mouse.[29] The methanolic extract of *I. frutescens* rind 400 mg/kg were administered orally 30 min prior to Carrageenan administration. The paw *edema size* was measured at 0, 1, 2, 3 & 4 hours by using dial caliper.[30] The percentage inhibition of paw *edema* in drug treated group was compared with the control group. Diclofenac sodium (5 mg/kg p.o.) was used as reference standard. 0 hour reading was considered as an initial normal paw size. Data was collected from the paw thickness and percentage inhibition of paw edema of the treated animals. Percentage inhibition of paw edema was calculated by using the formula.

Anti inflammatory activity (%) = (1-T/C) x100

Where T is the change of paw diameter in treated group and C is the change of paw diameter in control group.

Data Analysis: All values were expressed as mean ± Standard error of mean (SEM). Statistical comparison were performed by One-way analysis of variance (ANOVA), followed by using Dunnett test. Results were considered as significant of the differences between the test and control group data when p values less than 0.001, 0.01, 0.05 ($p<0.05$, $p<0.01$, $p<0.001$)

RESULT AND DISCUSSION

Table: 01 Results of Phytochemical Screening.

Tested groups	Methanolic Extract of *I. frutescens*
Carbohydrate	+
Glycoside	+
Alkaloid	-
Saponin	+
Protein	+
Flavonoid	+
Tannin	-
Steroid	+
Anthraquinon	-

Note: + = Indicates the presence of the tested group, - = Indicates the absence of the tested group.

Table: 02-Results of Analgesic effect of *I. frutescens* extract on acetic acid-induced writhing in mice

Animal Group	Writhing Counting (Mean ±SEM)
Control Group	39.5±0.87
Standard Group	6.75±0.43***
Extract Group (300 mg/kg)	14±1.15***

Values were Mean ± SEM, (n=4); $^{***}p<0.001$ Dunnett test as compared to Control Group.

Table: 03-Results of Anti-inflammatory effect of *I. frutescens* extract on carrageenan induced paw edema (mm) in mice.

Animal Group	60 min	120 min	180 min	240 min
Control	0.28±0.017	0.33±0.028	0.30±0.011	0.30±0.023
Standard	0.16±0.017**	0.13±0.005**	0.12±0.017***	0.11±0.005***
Extract(400mg)	0.21±0.017	0.19±0.023**	0.17±0.017**	0.14±0.017***

Experimental data were presented as mean ± SEM. By using the Dunnett test significant differences (*p<0.05, **p<0.01 and ****P*-value<0.001) between the means were determined compare to control group where n=04. For statistical evaluation IBM-SPSS software version 20 was utilized.

CONCLUSION

Natural products are a huge resource for medicine as shown with the use of plants in different pharmaceutical products. Therefore the investigation medicinal value of plants has become a matter of great significance. Particularly in preventing or treating serious health conditions such as Diabetes, cancer, acquired immune deficiency syndrome (AIDS), and hypercholesterolemia and against pain. Most significant analgesic activity was observed (p<0.05) in extract 300 mg/kg inhibit 64.56% of writhing reflex compared to standard drug diclofenac 82.91% writhing inhibition. Also a strong anti-inflammatory activity was observed (p<0.05) in extract 400 mg/kg inhibited paw volume 41.02% induced by 1% carrageenan compared with standard group 56.69% inhibition of paw volume. The present study indicates significant analgesic; anti-inflammatory effects of *I. frutescens.* In the light of our pharmacological studies of *I. frutescens* extract can be useful as analgesic and anti-inflammatory treatment. Further investigations, must be carried out to examine underlying mechanism of analgesic and anti-inflammatory effects of *I. frutescens*

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