

IN VIVO EVALUATION OF ANALGESIC, ANTI-INFLAMMATORY AND ANTI-DIABETIC ACTIVITIES OF METHANOL EXTRACT OF *CARALLIA BRACHIATA* L. LEAVES

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Abstract

Most of analgesic and anti-inflammatory drugs cause hyperacidity and peptic ulcer. The present study was carried out to evaluate analgesic, anti-inflammatory and anti-diabetic activities of methanol extract of *Carallia brachiata* L. leaves on experimental mice. *Carallia brachiata* L. leaves showed significant analgesic effects ($*p < 0.01$) on acetic acid induced writhing of mice compared to the control group where diclofenac sodium was used as standard. *Carallia brachiata* L. leaves decreased the carrageenan induced paw edema notably ($*p < 0.05$ and $**p < 0.01$) compare to control group in which standard was diclofenac sodium. The methanol extract of *Carallia brachiata* L. leaves was administered intraperitoneally as a single dose of 150 mg/kg body weight to alloxan induced diabetic rats and found to reduced blood lipid level (Total cholesterol and Triglycerides) significantly ($*p < 0.05$). The extract also exhibited correlation of altered biochemical parameters viz., SGOT and SGPT levels in diabetic rates. The effect of plant extract was compared with the standard drug metformin. The phytochemical screening tests indicated that the different constituents such as saponins, tannins, triterpenes, alkaloids and flavonoids etc. were present in the plant which has analgesic, anti-inflammatory and anti-diabetic properties but further study needed to evaluate the mechanism of action of antidiabetic activities of *Carallia brachiata* L.

Keywords: *Carallia brachiata* Leaf, Analgesic, Anti-inflammatory and Anti-diabetic activities.

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 an 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 [10-11]. 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 [12]. Prescription as well as over-the-counter NSAIDs except aspirin increases the risk of myocardial infarction and stroke [13]. 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 [14,15]. Diabetes, a metabolic disorder of multiple etiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. According to WHO in 2014, 9% of adults 18 years and older had diabetes. 1.5 million deaths worldwide occurs in 2012 due to diabetes and more than 80% of diabetes deaths occur in low- and middle-income countries [16,17]. In particular, type 2 diabetes mellitus (T2DM) is the most encountered form of diabetes, accounting for more than 80% of the total cases of diabetes [18,19]. Glucose metabolism disturbances are major factors leading to diabetes. The insulin released by the pancreatic β -cells is the hormone responsible for glucose homeostasis [20,21]. Insulin stimulates hepatocytes, myocytes, and adipocytes to uptake glucose from the circulatory system. Depending on need, glucose can either be used as an energetic source by glycolysis, or alternatively, stored as glycogen inside muscle or liver cells. The inappropriate utilization of insulin leads to insulin resistance, which is characterized by the inability of cells to respond to normal levels of circulating insulin [19], thus leading to the occurrence of the disease.

Carallia brachiata is a most important plants in terms of medicinal properties. It is widely distributed in tropical Asia, Indomalaysia and Australia. This plant grows up to 25 m tall. Traditionally it is used for the treatment of fever, small pox and itch etc [22]. The

aim in this study is to evaluate the analgesic, anti-inflammatory and antidiuretic effect of methanolic extract of *Carallia brachiata*.

Methods

Plant material

The leaves of the plant *Carallia brachiata* were collected from the Dhaka, Bangladesh and identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka Bangladesh for its authentication.

Preparation of the extract:

The harvested leaves of the plant were separated from undesirable materials. They were air dried for two weeks after cutting into small pieces. The parts of the plant were ground into a coarse powder with the help of a suitable grinder. The powder was put away in a airtight sealed compartment and kept in a cool, dim and dry spot. Then, the plant powder will dissolve in methanol solution in a flat bottom sealed container for several days. Then metabolic extract were evaporates by the rotary evaporator and plant extract were collect.

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. The standard drug, Metformin hydrochloride was the generous gift samples from Beximco Pharmaceuticals Ltd of Bangladesh. Blood samples analyzed for blood glucose content by using OK meter Match glucose test meter (Hsinchu, Taiwan). Alloxan, DPPH (1,2-diphenyl-2-picrylhydrazyl), Ascorbic acid and other solvents were collected from laboratory of Bangladesh University. All chemicals that were used in the research were analytical graded.

Experimental animals:

Eight week-old Swiss 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.

Phytochemical screening: Various phytochemical tests which were performed under the heading of phytochemical screening are Benedict's Test and Fehling's Test for carbohydrates, general test for glycosides, tests for alkaloids by Mayer's reagent and Dragendroff's reagent, test for Saponins, test for Flavonoids, test for Steroids and test for gums [23].

Detection of carbohydrates:

Extracts were dissolved independently in 5 ml refined water and separated. The filtrates were utilized to test for the presence of carbohydrates.

Benedict's Test: Filtrates were treated with Benedict's reagent and warmed tenderly. Orange red encourage shows the presence of reducing sugars.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and warmed with Fehling's A and B solutions. Development of red precipitate shows the presence of reducing sugars.

Tests for Glycosides: 2 ml solution of the extract was taken into a test tube. 1ml mixture of Fehling solution A and B then added into the test tube. The tube was set in a water-bath at 60° C. On the off chance that a brick red ppt. structure that demonstrates the presence of glycosides.

Test for alkaloids:

Mayer's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. At that point 1 ml of Mayers reagent was included. Yellow color ppt. was shaped and that was shown as the presence of alkaloids.

Dragendroff's test: 2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. At that point 1 ml of Dragendroff's reagent was included. Orange brown colored precipitate was framed and that was demonstrated as the presence of alkaloids.

Test for Steroids:

Sulphuric acid test: 1 ml solution of chloroform extract was taken and then added 1ml Sulphuric acid Red color indicates the presence of steroid.

Test for gums: 5 ml solution of the extract was taken and after that molish reagent and sulphuric acid were included. Red violet ring created at the intersection of two fluids demonstrated as the presence of gums and carbohydrate.

Test for Flavonoids: Included a couple of drops of concentrated hydrochloric acid to a little measure of a alcoholic extract of the plant material. Prompt improvement of a red color demonstrates the presence of Flavonoids.

Test for Saponins: 1 ml solution of the extract was diluted with refined water to 20 ml and shaken in a graduated cylinder for 15 minutes. One centimeter layer of foam indicates the presence of saponins.

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 *Carallia brachiata* L. extract Group (received 250mg/kg & 500mg/kg *Carallia brachiata* L. 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 1% 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 [24]. Percentage protection of acetic acid induced writhing was calculated by the formula.

$$\text{Percentage protection} = (Wc - Wt) / Wc \times 100.$$

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 [25]. The methanolic extract of *Carallia brachiata* L. rind 250 mg/kg & 500 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 [26]. The percentage inhibition of paw edema in drug treated group was compared with the control group. Diclofenac sodium (10 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.

$$\text{Anti-inflammatory activity (\%)} = (1 - T/C) \times 100$$

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

Anti-diabetic activity

Preparation of standard drug

5gm of Metformin were obtained from Beximco pharmaceuticals. Then the powder was dissolved in 25.70ml of distilled water to get the stock solution of 19.20g/dl.

Induction of Diabetes mellitus

The animal were weighted and injected via intraperitoneal route: 90 mg/kg of Alloxan dissolved in normal saline using insulin syringes. Diabetes mellitus was confirmed after 72 hr of alloxan injection by testing the fasting glucose level in the blood obtained from the tail vein of the animals using glucometer and glucose tests strip. The result of the blood glucose measurement by glucometer correlates excellently well with the result obtained from the laboratory methods [27].

Experimental design

Sixteen (16) Swiss albino mice were grouped into four (4) different groups containing 4 mice in each group. All the groups were kept into different cages. The groupings of the mice were done by as follows:

1. Group I: Diabetic Control, these mice were induced diabetes but not given any form of treatment throughout the experiment procedure.
2. Group II: Diabetic Standard (Standard Group, Metformin HCl, 100 mg/kg), the rats were induced with diabetes and treated with a standard drug (Metformin) at a dose of 100mg/kg.
3. Group III: Diabetic Extract (Extract Group 250 mg/kg), the mice were induced with diabetes and treated with low dose of *Carallia brachiata* L. leaves extracts.
4. Group IV: Diabetic Extract (Extract Group 500 mg/kg), the mice were induced with diabetes and treated with higher dose of *Carallia brachiata* L. leaves extracts.

Administration of drugs

Both Metformin and *Carallia brachiata* L. were administered via the oral route with the aid of an oropharyngeal cannula. The mice were handled appropriately to restrict movement and prevent trauma to the mice during drug administration.

Measurement of fasting blood glucose level

All mice after 1 hour of feeding of extract and/ drug and three more blood samples were collected at 30, 60, 90 and 120 minutes intervals and blood glucose level was estimated in all the experiments by using glucometer.

Data Analysis: All values were expressed as mean \pm 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 0.01 and 0.05 ($p < 0.05$, $p < 0.01$).

Results

Phytochemical screening

The phytochemical screening tests indicated that the different constituents such as alkaloids, tannins, glycosides and saponins were present in the plant *Carallia brachiata* which have pharmacological properties. The results of various qualitative chemical tests for the detection of chemical constituents of *Carallia brachiata* is shown in the Table 1.

Analgesic effect

This condition was markedly reduced by Diclofenac (82.22%) as well as the extract, with the effect observed at 250 mg/kg (45.45%) and 500 mg/kg (60.24%) as shown in Table 2. In addition, the extract significantly reduced the frequency of defecation and the number of rying when compared with control. Percentage of rying output was also reduced by doses of the extract.

Anti-inflammatory activity

Carrageenan induced paw edema test: To the carrageenan (10 mg/mL) induced paw edema rats, the MeOH extract, at the dose of 250 and 500 mg/kg, body wt. exerted a significant ($p < 0.05$; $p < 0.005$) and dose dependent inhibition on paw edema compared to the control group (Table 3). All of the fractions exhibited prominent anti inflammatory effect.

Effect of different fractions of *Carallia brachiata* on fasting blood glucose (FBG) level in the glucose-induced hyperglycemic mice

In case of glucose-induced hyperglycemia, standard drug (metformin) reduced blood glucose level to 90%, 82%, 68% and 59% in 60, 120, 180 and 240

minutes, respectively. So, metformin caused maximum reduction of blood glucose level of 59% in 240 minutes. *Carallia brachiata* (250 mg/kg) fraction reduced blood glucose level to 77%, 74%, 68% and 68% in 60, 120, 180 and 240 minutes, respectively. *Carallia brachiata* (500 mg/kg) fraction reduced blood glucose level to 67%, 63%, 57% and 50% in 60, 120, 180 and 240 minutes, respectively as shown in the table 4. Here the plant fraction showed a dose dependent effect and maximum reduction of 50% was observed by *Carallia brachiata* (500 mg/kg) fraction in 240 minutes (Table 4). The results were compared with normal control group which received glucose only.

Discussion

Differing group of phenolic compound in plant have a perfect basic science with the expectation of complimentary radical searching action by exhibiting a wide range of physiological properties, such as anti-allergeni, anti-inflammatory, anti-microbial and vasodilatory effects etc. [28, 29]. The phytochemical screening tests indicated that the different constituents such as saponins, tannins, triterpenes, alkaloids and flavonoids etc (Table 1). Plant extract inhibits pain with the both mechanism, proposing that the plant extract may act as pain relieving agent [30]. In the present study strong analgesic effect of methanol extract was found in doses of 500 mg/kg & 250 mg/kg and the results were 60.00% & 45.45% inhibition of writhing respectively compared to the standard drug Diclofenac-Na 82.91 % writhing inhibition. For its analgesic potentialities it may be used as a traditional medicine but further study is needed to evaluate the mechanism of action of analgesic activity and toxicity study of *Carallia brachiata* (Table 2).

The brain and spinal cord assume a noteworthy job in central pain mechanism. The dorsal horn of the spinal cord is enriched with a few neurotransmitters and receptors which are the significant focuses for pain and inflammation [31]. Narcotic analgesics restrain both peripheral and focal system of pain, while non steroidal anti-inflammatory drugs inhibit only peripheral pain [32]. Since the extract/fractions significantly inhibited paw edema induced by carrageenan in the second phase and this finding suggests a possible inhibition of cyclooxygenase

synthesis by the extract and this effect is similar to that produced by non-steroidal anti-inflammatory drugs such as indomethacin, whose mechanism of action is inhibition of the cyclooxygenase enzyme. Furthermore, it has observed that polyphenols (including phenolic compounds and flavonoids) rich plant extracts possess antioxidant as well as anti-inflammatory activity [28, 33]. In the current study, strong anti-inflammatory effect was observed of methanol extract in doses of 250 mg/kg & 500 mg/kg and the results were 71.25% and 46.51% inhibition of paw edema respectively compared to the standard drug Diclofenac-Na 82.30% inhibition of paw edema (Table 3). For its mentionable anti-inflammatory potentialities it may be used as a traditional medicine but for the definite source of drug more investigations must be carried out to evaluate the mechanism of action of anti-inflammatory effects of *Carallia brachiata*.

The pathogenesis of diabetes mellitus and the possibility of its management by existing therapeutic agents without any side effects have stimulated great interest in recent years. Management of diabetes without any side effects is still a challenge for the medical system. This leads to an increasing search for improved antidiabetic drugs. Few of plant treatments used in traditional medicine for diabetes have received scientific scrutiny [34]. Oral glucose tolerance test (OGTT) measures the body's ability to use glucose, the body's main source of energy [35]. In this study, the extract of *Carallia brachiata* leaves showed a remarkable decrease of fasting blood glucose level from 60 min to 240 min in a dose dependent manner. For its blood glucose level lowering potentialities it may be used as a traditional medicine but further study needed to evaluate the mechanism of action of antidiabetic activities of *caralliabrachiata*L. The fractions of plant extract enhanced glucose utilization. So the blood glucose level was significantly reduced in the glucose loaded rats. This may be due to the presence of hypoglycemic saponins, tanins, triterpenes, alkaloids and flavonoids etc. [Table 4].

Conclusion

The plant *Carallia brachiata* exhibited promising effect for analgesic anti-inflammatory and oral glucose tolerance activity. Now it can be concluded on the basis of results obtained from investigation

that the plant may be useful as analgesic, anti-inflammatory as well as antidiabetic drug (crude drug). However, further study is needed to evaluate the mechanism of action of analgesic, anti-inflammatory and antidiabetic activity and toxicity study of *Carallia brachiata*.

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Table 1. Results of Phytochemical Screening.

| Tested groups | Methanol Extract of <i>Carallia brachiata</i> L. |
|---------------|--|
| Glycoside | + |
| Alkaloid | + |
| Flavonoid | - |
| Tannin | + |
| Saponins | + |

(+) = Present; (-) = Absent

Table 2. Results of Analgesic effect of *Carallia brachiata* L. extract on acetic acid-induced writhing in mice

| Animal Group | Writhing Counting (Mean) | % of Writhing Inhibition |
|------------------------------------|--------------------------|--------------------------|
| Control Group | 45 | - |
| Standard Group (Diclofenac Sodium) | 08 | 82.22 |
| Methanol extract Group (250 mg/kg) | 25 | 45.45 * |
| Methanol extract Group (500 mg/kg) | 18 | 60.24 ** |

Values are mean±SEM, (n = 5); *p<0.05, **p<0.01, student's t-test compared to control.

Table 3. Results of Anti-inflammatory effect of *Carallia brachiata* L. extract on carrageenan induced paw edema (mm) in mice.

| Animal Group | 60 min | 120 min | 180 min | 240 min |
|--------------------------------------|--------|---------|---------|---------|
| Control | 4.4 | 4.38 | 4.23 | 4.17 |
| Standard | 4.35 | 3.87 | 3.5 | 3.05 |
| <i>Carallia brachiata</i> (250mg/mL) | 4.22 | 4.15 | 4.11 | 4.02 |
| <i>Carallia brachiata</i> (500mg/mL) | 4.75 | 4.5 | 4.17 | 3.55* |

Values are mean±SEM, (n = 5); p<0.05, student's t-test compared to control.

Table 4. Results of *Carallia brachiata* L. extract on fasting blood glucose (FBG) level in the glucose-induced hyperglycemic mice

| Animal Group | 0 min | 60 min | 120 min | 180 min | 240 min |
|---------------------------------------|-------|--------|---------|---------|---------|
| Control Group (mM/L) | 23.03 | 22.40 | 22.10 | 22.30 | 21.01 |
| Standard Group (mM/L) | 22.50 | 20.25 | 18.21 | 15.20 | 12.34 |
| <i>Carallia brachiata</i> (250 mg/kg) | 22.84 | 17.27 | 16.35* | 15.12* | 14.27* |
| <i>Carallia brachiata</i> (500 mg/kg) | 22.38 | 15.12* | 13.97** | 12.65** | 10.52** |

Values are mean±SEM, (n = 5); *p<0.05, **p<0.01, student's t-test compared to control.