

HYPOGLYCEMIC AND ANALGESIC ACTIVITY OF ETHANOL EXTRACT OF POLYALTHIA LONGIFOLIA LEAVES ON EXPERIMENTAL MICE

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Abstract

Diabetes is emerging new threats through different complications with time. Present study was designed to evaluate a hypoglycemic activity of Polyalthia longifolia leaves ethanol extract of along with analgesic activity. Oral glucose tolerance test showed significant result at dose 500mg/kg body weight of mice but not significant at dose 250mg/kg compare to control group where metformin was used as a reference standard. Analgesic activity of Polyalthia longifolia extract by acetic acid induced writhing method also showed mentionable effects that was 58% at dose 500mg/kg and 70% at 250mg/kg compare with standard diclofenac 78% writhing inhibition.

Keywords: Hypoglycemic, Analgesic Activity, Acetic Acid, Glucose tolerance, Polyalthia Longifolia

Introduction

Due to the increasing development of drug resistance in human pathogens as well as the appearance of undesirable effect on certain antimicrobial agents, there is a need to search for new agents. The world health organization in 1997 suggested that effective locally available plants be used as substitutes for drugs. Research work on medicinal plants be intensified and information on these plants be exchanged. This thought will go a long way in the scientific exploration of medicinal plants for the benefit of man and is likely to decrease the dependence on importance of drugs [1]. *Polyalthia longifolia* (Annonaceae) is a tree, which is widely distributed in Bangladesh, Srilanka and throughout the hotter parts of India [2]. In India, the seeds of this plant were used as febrifuge [3]. Literature survey revealed that most of the plants of annonaceae family contain antitumor and anticancer principles [4,5]. The bark is also used as a febrifuge in the Balasore district of Orissa [6]. The extract of stem bark and the alkaloids isolated from this were found to demonstrate a good antibacterial and antifungal activities [7]. In the present study, antimicrobial potentiality of the *Polyalthia longifolia* leaves was investigated against a few clinically isolated as well as standard microbial cultures.

Diabetes mellitus (DM) is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretion and/or insulin action [8]. It is one of the common metabolic disorders with micro- and macrovascular complications that results insignificant morbidity and mortality. It is considered as one of the five leading causes of death in the world [9]. There are an estimated 143 million people in the world with diabetes mellitus and this number will probably double by the year 2030. A medicinal plant, *Polyalthia longifolia*, led to the discovery and synthesis of metformin [10]. Despite considerable progress in the treatment of diabetes by oral hypoglycemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations. In recent times, there has been a renewed interest in the plant remedies [11-12].

It is having Javarashaka (reducing fever) action. Bark is useful in fever. In some places, it is a substitute for Ashoka (*Saraca ashoka*) bark, but it is not advisable. The bark is bitter, acrid, cooling, febrifuge, and anthelmintic. It is useful in fever, skin disease, diabetes, hypertension, helminthiasis, and vitiated conditions of vata and pitta. [13]

Methods

Plant Materials

Polyalthia longifolia leaves were collected from in front of the "National Parliament House", Bangladesh during the month of January, 2011 and the plant authenticity was confirmed from the Bangladesh National Herbarium, Mirpur, Dhaka (Accession Number: DACB – 35392).

Preparation of Plant Extracts

The collected leaves were separated from undesirable materials of plant parts. The leaves of *Polyalthia longifolia* were shade dried for fifteen days at room temperature to ensure the active constituents free from decomposition. The dried leaves were powdered in an electrical grinder after overnight drying in an oven below 50°C. The powder was extracted with 96% ethanol at room temperature. The bottle were kept at room temperature and allowed to stand for several 7-10 days with occasional shaking and stirring. When the solvent become concentrated, the liquid alcohol contents were filtered through cotton and then through filter paper (Whatman Fitter Paper No. 1). Finally, a highly concentrated ethanolic extract were obtained.

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 *Polyalthia longifolia*.fruit extract Group (received 300mg/kg *Polyalthia longifolia* fruit 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 10 minutes.^[14] Percentage protection of acetic acid induced writhing was calculated by the formula.

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.

Method for Evaluation of Hypoglycemic Activity

Experimental Animals

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

Induction of Diabetes

After fasting 16h, diabetes was induced into mice by intra-peritoneal injection (i. p.) of alloxan monohydrate (100 mg/kg), dissolved in saline (100 µl/mice, ip.). After 48h, plasma glucose levels were measured by glucometer (Tyson, Taiwan) using a blood sample from tail-vein of mice. Mice with blood sugar level higher than 08.5-11.5 mmol/l are considered as diabetic. Age-matched healthy mice were used to examine the effects of extracts on normal mice.

Experimental Protocols

After induction of diabetes 20 mice were divided into five groups for the oral administration either-

- I. Normal Control (Normal Group, Vehicle 0.5% methyl cellulose)
- II. Diabetic Control (Control Group, Vehicle 0.5% MC)
- III. Diabetic Standard (Standard Group, Metformin HCl, 100mg/kg, op)
- IV. Diabetic + Extract (250mg Group, 250mg/kg)
- V. Diabetic + Extract (500mg Group, 500mg/kg)

For analgesic test all mice were divided into four groups. Each group comprises 4 mice (n = 4). Control group (received 0.5% methyl cellulose), Standard Group (received Diclofenac 75mg, 1ml), 250mg Group (received 250mg/kg extract) and 500mg Group (received 500mg/kg extract).

Oral Glucose Tolerance Test (OGTT) in diabetic mice

The mice were fasted over-night and next day blood samples were taken from all groups of animals to estimate fasting blood glucose level (0 min). All mice received 1g /kg glucose, after 1 hour of feeding of extracts and/ drug and three more blood samples were collected at 30, 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 ± 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 (p<0.001)

Results

Table 1: Effects of the ethanolic extracts of leaf of *Polyalthia longifolia* on acetic acid-induced writhing in mice.

Values are mean ± SEM, (n=5); ***:p<0.001, Dunnett test as compared to control group. Control Group animal received vehicle (1% Tween 80 in water), Standard Group received Diclofenac 75 mg/ kg body weight, 250mg Group and 500mg Group were treated with 250 and 500mg/kg body weight (p.o) of the crude extract of *Polyalthia longifolia*. Table 1 shows the effects of the extract of an acetic acid-induced writhing in mice. The oral administration of both doses of *Polyalthia longifolia* extract significantly (p<0.001) inhibited writhing response induced by acetic acid in a dose dependent manner compared. The effect was dose dependent and the most significant effect observed with 500mg Group (500 mg/kg) which is very close to the standard group compared to control group.

Table 2 Effect of the ethanolic extract of *Polyalthia longifolia* (leaf) on oral glucose tolerance test in diabetic mice.

Values were expressed in Mean ± SEM value. Each group comprised 5 animals. Control group received 0.5% Methyl cellulose and standard group received 100mg/kg Metformin. After oral administration of glucose the blood glucose levels were significantly higher in diabetic control and experimental groups of mice as shown in Table-2 and Figure-3. In diabetic control the peak increase in blood glucose

concentration was observed after 30 min and remained high over the next hour. Mice treated with extract in Group (250mg/kg and 500mg/kg), showed significant decrease (from 16.45mM \pm SEM to 12.55 \pm SEM) and (from 16.25mM \pm SEM to 6.22mM \pm SEM) (* p <0.05) in blood glucose concentration at 90 min and 120 min compared with diabetic control. mice (from 14.82mM \pm SEM to 14.97mM \pm SEM). Prominent effects were observed with Extract Group (250mg/kg and 500mg/kg) and this effect is like that of Standard Group (from 15.62mM \pm SEM to 4. 87mM \pm SEM).

Discussion

Diabetes mellitus is one of the most common chronic disease and is associated with hyperglycemia, polyurea, polydipsia, polyphagia, weight loss, muscle weakness, hyperlipidemia and co-morbidities such as obesity, hypertension. Hyperglycemia and Hyperlipidemia are the two metabolic complications of both clinical and experimental diabetes (Bierman EL 1975).

Alloxan, a β -cytotoxin, induces "chemical diabetes" (alloxan diabetes) in a wide variety of animal species by damaging the insulin secreting pancreatic β -cell, resulting in a decrease in endogenous insulin release, which paves the ways for the decreased utilization of glucose by the tissues (Omamoto H 1981).

In the light of the literature on *Polyalthia longifolia*, we made an attempt for the first time to study the effect of *Polyalthia longifolia* extract in hyperglycemic mice. The experiment showed that, the extracts have the properties to stimulate or regenerate the β -cell for the secretion of insulin and are most effective for controlling diabetes by various mechanisms due to presence of hypoglycemic alkaloids, saponins and flavonoids.

Oral Glucose Tolerance Test (OGTT) measures the body ability to use glucose, the body's main source of energy (Du Vigneaud 1925). It can be used to diagnose prediabetes and diabetes. In our study, it is found that various fractions have also hypoglycemic effect in glucose induced hyperglycemic mice. The effects of extracts on blood sugar levels are dose dependent.

Induction of diabetes with alloxan was associated with decrease in hepatic glycogen, which could be attributed to the decrease in the availability of the

active form of enzyme glycogen synthetase probably because of low levels of insulin (A.H.Gold 1970, R.K.Goel 2004). In the present study, *Polyalthia longifolia* restored the depressed hepatic glycogen levels possibly by increasing the level of insulin. Our result showed that supplementation of diabetic mice with plant extracts resulted in significant elevation in hepatic glycogen content.

Acetic acid-induced writhing model represent pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from phospholipids. The acetic acid-induced writhing model response is a sensitive procedure to evaluate peripherally acting analgesic. The response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway (Voilley 2004, Hossain 2006). Preliminary photochemical screening reveals the presence of flavonoid, alkaloids, tannins and saponins in the plant extract. So the observed analgesic activity may be attributed to these compounds.

Further studies is required for the detailed studies pharmacological investigations of the leaf constituents, which have many pharmacological activity reported traditionally and its exact mechanism of action.

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Table 1: Effects of the ethanolic extracts of leaf of *Polyalthia longifolia* on acetic acid-induced writhing in mice.

Animal Group	Writhing Counting (Mean \pm SEM)
Control Group	41.75 \pm 1.70
Standard Group	9.5 \pm 1.29
250mg Group	17.5 \pm 2.08***
500mg Group	12.75 \pm 1.70***

Table 2: Effect of the ethanolic extract of *Polyalthia longifolia* (leaf) on oral glucose tolerance test in diabetic mice.

Time	Normal Group	Control Group	Standard Group	250mg Group	500mg Group
0Min	5.65 \pm 0.38	14.82 \pm 1.79	15.62 \pm 1.31	16.45 \pm 0.75	16.25 \pm 0.88
30Mins	5.65 \pm 0.38	15 \pm 1.47	6.07 \pm 0.56*	14.82 \pm 0.62	13.5 \pm 0.80
90Mins	5.86 \pm 0.52	15.12 \pm 1.43	4.97 \pm 0.17*	14.35 \pm 0.50	7.02 \pm 0.85*
120Mins	5.82 \pm 0.22	14.97 \pm 1.74	4.87 \pm 0.15*	12.55 \pm 0.42	6.22 \pm 0.41*