

EFFECT OF METHANOLIC EXTRACT OF *ANANAS COMOSUS* LEAVES ON GLUCOSE TOLERANCE AND ACETIC ACID-INDUCED PAIN IN SWISS ALBINO MICE

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ABSTRACT

In oral glucose tolerance test (OGTT), methanol extract of *Ananas comosus* leaves, when administered orally to glucose-loaded Swiss albino mice at doses of 50, 100, 200 and 400 mg/kg body weight led to dose-dependent reductions in blood glucose levels. At these doses, the percent reductions in blood glucose levels were, respectively, 6.5, 20.2, 30.5, and 46.9% compared to control animals. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg/kg body weight reduced blood glucose levels by 47.3%. The extract at the afore-mentioned four doses, also dose-dependently reduced acetic acid-induced abdominal constrictions in mice by 29.6, 37.0, 44.4, and 48.1%, respectively. A standard analgesic drug, aspirin, when administered at doses of 200 and 400 mg/kg reduced the number

of abdominal constrictions, respectively, by 44.4 and 55.6%. Preliminary phytochemical screening showed presence of alkaloids, flavonoids, saponins and tannins in the extract, which components can be responsible for the observed blood glucose lowering and analgesic effects.

KEY WORDS: *Ananas comosus*, OGTT, antihyperglycemic, analgesic.

INTRODUCTION

Ananas comosus (L.) Merr. Is a plant belonging to the Bromeliaceae family and which is cultivated in many parts of the world including Bangladesh for its edible fruits. The leaves are tough and are discarded in Bangladesh, but in the Philippines the pineapple's leaves are used to produce the textile fiber piña. The fruit as well as the plant is known in English as pineapple, and in Bangladesh as anarosh. The whole plant is used to treat typhoid fever in Ijebu Ode Local Government Area in Ogun State, Nigeria. ^[1] The fruits are used to treat convulsions by herbal practitioners in Yagba East Local Government of Kogi State, Nigeria. ^[2] Roasted unripe fruit juice is taken by different communities of Gohpur of Sonitpur district, Assam, India for strangury. ^[3] Fruit juice is taken to add on more weight by the various ethnic communities of Oyo State in Nigeria. ^[4] The Garo tribal community living in Netrakona district of Bangladesh use fruit juice for fever and leaf juice for helminthiasis and jaundice. ^[5]

Reported phytochemical constituents in the plant (excluding fruits) include 2,5-dimethyl-4-hydroxy-3(2H)-furanone, 5-hydroxytryptamine, acrylic acid, ananasic acid, beta-methyl-thiopropionic acid methyl ester, beta-methyl-thiopropionic acid ethyl ester, ergosterol peroxide, and stigmast-5-ene-3-beta-7-alpha-diol. ^[6] Seven compounds, ananasate, 1-O-caffeoylglycerol, 1-O-p-coumaroylglycerol, caffeic acid, p-coumaric acid, beta-sitosterol and daucosterol have been reported from leaves of the plant. ^[7] Ethanolic extract of leaves containing a mixture of alkaloids, flavonoids, tannins, phytosterols, glycosides and phenols have been reported to lower blood glucose levels in streptozotocin (STZ)-induced diabetic rats. ^[8]

Fruit juice demonstrated a synergistic action with glimepiride in lowering blood glucose levels in alloxan induced diabetic rats. ^[9] Fruit residue of the plant was assessed for antidiabetic potential; a number of compounds like sinapic acid, daucosterol, 2-methylpropanoate, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, methyl 2-methylbutanoate and triterpenoid ergosterol were found to be responsible for the observed antidiabetic effects. ^[10] Ethanolic extract of leaf juice reportedly increased sensitivity to insulin in STZ-treated diabetic rats and HepG2 cells. ^[11] Ethanol extract of leaves reportedly reduced blood glucose levels in alloxan diabetic rats but not in normal rats in oral glucose tolerance tests (OGTT). ^[12] Antiinflammatory and analgesic effects have been reported with oral administration of bromelain (isolated from fruits and stems) in acetic acid induced writhing tests in mice and carragennin induced paw inflammation in rats. ^[13]

Diabetes and pain are common afflictions in Bangladesh. The rural people suffering from diabetes or pain arising from a variety of causes often lack access or cannot afford modern antidiabetic and analgesic medications. On the other hand, they can easily avail themselves of medicinal plants growing in their vicinity or plants that are being cultivated by them. It is also a fact that newer drugs are necessary for controlling diabetes and relieving pain, for existing antidiabetic drugs may be too costly and analgesic, even over-the-counter drugs like aspirin and paracetamol, produce unwanted side-effects like gastric ulceration or hepatotoxicity. Towards remedying this problem, we had been systematically screening both common and uncommon plants of Bangladesh for their antihyperglycemic and analgesic potential.^[14-25] It appears from previous reports that the various plant parts of *A. comosus* may have antihyperglycemic and analgesic potential and the responsible phytoconstituents. The objective of the present study was to evaluate these two potentials in methanolic extract of leaves through oral glucose tolerance tests in glucose-loaded mice and intraperitoneally acetic acid-induced pain model in mice.

METHODS

Plant Material Collection

Leaves of *A. comosus* were collected during June 2013 from a local market in Dhaka city, Bangladesh, and taxonomically identified at the Bangladesh National Herbarium (Accession Number 38,441).

Preparation of Methanolic Extract of Leaves

Leaves were cut into small pieces, air-dried in the shade, and 150g of dried and powdered leaves were extracted with methanol (w: v ratio of 1:5, final weight of the extract 3.99g).

CHEMICALS AND DRUGS

Glibenclamide, aspirin, and glucose were obtained from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

Animals

Swiss albino mice, which weighed between 15-20g, were used in the present study. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). The animals were acclimatized for three days prior to actual experiments. The study was conducted following approval by the Institutional Animal Ethical Committee of University of Development Alternative, Dhaka, Bangladesh.

Oral Glucose Tolerance Tests for Evaluation of Antihyperglycemic Activity

Oral glucose tolerance tests were carried out as per the procedure previously described by Joy and Kuttan (1999) ^[26] with minor modifications. Briefly, fasted mice were grouped into six groups of five mice each. The various groups received different treatments like Group 1 received vehicle (1% Tween 80 in water, 10 ml/kg body weight) and served as control, Group 2 received standard drug (glibenclamide, 10 mg/kg body weight). Groups 3-6 received methanolic leaf extract of *A. comosus* (MEAC) at doses of 50, 100, 200 and 400 mg per kg body weight. All substances were orally administered. Following a period of one hour, all mice were orally administered 2g glucose/kg of body weight. Blood samples were collected 120 minutes after the glucose administration through puncturing heart. Blood glucose levels were measured by glucose oxidase method. ^[27] The percent lowering of blood glucose levels were calculated according to the formula described below.

Percent lowering of blood glucose level = $(1 - W_e/W_c) \times 100$,

Where W_e and W_c represents the blood glucose concentration in glibenclamide or MEAC administered mice (Groups 2-6), and control mice (Group 1), respectively.

Analgesic Activity Evaluation through Abdominal Writhing Test

Analgesic activity of MEAC was examined as previously described. ^[28] Mice were divided into seven groups of five mice each. Group 1 served as control and was administered vehicle only. Groups 2 and 3 were orally administered the standard analgesic drug aspirin at doses of 200 and 400 mg per kg body weight, respectively. Groups 4-7 were administered MEAC at doses of 50, 100, 200 and 400 mg per kg body weight, respectively. Following a period of 60 minutes after oral administration of standard drug or MEAC, all mice were intraperitoneally injected with 1% acetic acid at a dose of 10 ml per kg body weight. A period of 5 minutes was given to each animal to ensure bioavailability and onset of chemically induced irritation of acetic acid ^[29], following which period, the number of abdominal constrictions (writhings) was counted for 10 min. The percent inhibitions of abdominal constrictions were calculated according to the formula given below.

Percent inhibition = $(1 - W_e/W_c) \times 100$

Where W_e and W_c represents the number of writhings in aspirin or MEAC administered mice (Groups 2-7), and control mice (Group 1), respectively.

Acute Toxicity Test

It was of interest to determine the toxicity of the extract (MEAC). Acute toxicity test was conducted as previously described.^[30] Mice were divided into nine groups, each group consisting of six animals. Group 1 was given 1% Tween 80 in normal saline (2 ml per kg body weight). The other eight groups (Groups 2-9) were administered, respectively, 100, 200, 300, 600, 800, 1000, 2000 and 3000 mg of MEAC per kg body weight. All animals were closely observed for the next 8 hours to notice any behavioral changes or mortality and were kept under close observation for the next two weeks.

Statistical Analysis

Experimental values are expressed as mean \pm SEM. Independent Sample t-test was carried out for statistical comparison. Statistical significance was considered to be indicated by a p value < 0.05 in all cases.^[21]

Preliminary Phytochemical Screening

Preliminary phytochemical analysis of MEAC for presence of saponins, tannins, alkaloids, and flavonoids were conducted as described before.^[31]

RESULTS AND DISCUSSION

Toxicity Evaluation

The crude extract (MEAC) did not show any mortality in mice even at the highest dose tested. However, at doses of MEAC exceeding 2000 mg/kg, a slight demonstration of irritability was noted in the first 24 hours after dosing.

Preliminary Screening of Phytochemicals

Various tests conducted for presence of phytochemicals in MEAC indicated the presence of alkaloids, flavonoids, saponins, and tannins.

Antihyperglycemic Activity Evaluation Results

MEAC, when administered to mice at doses of 50, 100, 200 and 400 mg/kg demonstrated distinct improvements in glucose tolerance. The antihyperglycemic potential of MEAC was demonstrated through reductions of blood glucose in glucose-loaded mice by 6.5, 20.2, 30.5, and 46.9%, respectively, at the afore-mentioned four doses. A standard antihyperglycemic drug, glibenclamide, when administered at a dose of 10 mg/kg, reduced blood glucose concentration by 47.3%, compared to control mice. Thus at the highest dose tested (400

mg/kg), MEAC showed comparable antihyperglycemic results to that of glibenclamide. The results are shown in Table 1.

Table 1: Effect of Crude Methanol Extract of *A. Comosus* Leaves (MEAC) on Blood Glucose Level In Hyperglycemic Mice Following 120 Minutes of Glucose Loading.

Treatment	Dose (mg/kg body weight)	Blood glucose level (mmol/l)	% lowering of blood glucose level
Control	10 ml	5.24 ± 0.28	-
Glibenclamide	10 mg	2.76 ± 0.34	47.3*
(MEAC)	50 mg	4.90 ± 0.31	6.5
(MEAC)	100 mg	4.18 ± 0.15	20.2*
(MEAC)	200 mg	3.64 ± 0.30	30.5*
(MEAC)	400 mg	2.78 ± 0.37	46.9*

All administrations were made orally. Values represented as mean ± SEM, (n=5); **P* < 0.05; significant compared to hyperglycemic control animals.

Analgesic Activity Evaluation Results

Dose-dependent and significant reductions in the number of abdominal constrictions induced by intraperitoneal administration of acetic acid were observed with MEAC. At doses of 50, 100, 200 and 400 mg per kg body weight, MEAC was observed to reduce the number of constrictions, respectively, by 29.6, 37.0, 44.4, and 48.1%. A standard analgesic drug, aspirin, when administered to experimental animals at doses of 200 and 400 mg per kg body weight, reduced the number of constrictions by 44.4 and 55.6%, respectively. Thus, a dose of 200 mg/kg MEAC was equivalent to that of 200 mg/kg aspirin, and a dose of 400 mg MEAC/kg was better than that obtained with 200 mg/kg aspirin in reducing pain. The results are shown in Table 2.

Table 2: Analgesic Effect of Crude Methanol Extract of *A. Comosus* Leaves (MEAC) In Acetic Acid-Induced Pain Model Mice.

Treatment	Dose (mg/kg body weight)	Mean number of abdominal constrictions	% inhibition
Control	10 ml	5.4 ± 0.24	-
Aspirin	200 mg	3.0 ± 0.55	44.4*
Aspirin	400 mg	2.4 ± 0.40	55.6*
(MEAC)	50 mg	3.8 ± 0.66	29.6*
(MEAC)	100 mg	3.4 ± 0.51	37.0*
(MEAC)	200 mg	3.0 ± 0.32	44.4*
(MEAC)	400 mg	2.8 ± 0.20	48.1*

All administrations (aspirin and extract) were made orally. Values represented as mean ± SEM, (n=5); **P* < 0.05; significant compared to control.

The presence of alkaloids, flavonoids, saponins and tannins were observed in the extract (MEAC). In a previous report, ethanolic extract of leaves of *A. comosus* containing alkaloids, flavonoids, tannins and other phytochemicals has been observed to lower blood glucose levels in STZ-diabetic rats. ^[8] Thus these groups of phytochemicals can be responsible for the blood glucose lowering effect as observed in oral glucose tolerance tests in the present study. The antihyperglycemic and analgesic effects of alkaloids, flavonoids, saponins and tannins have also been noted with extract of other plants. The analgesic activity of *Aconitum baikalnensis* has been attributed to diterpene alkaloids. ^[32] Stem bark extract of *Tamarindus indica* reportedly demonstrated antihyperglycemic activity in alloxan diabetic rats; phytochemical screening of the extract showed the presence of glycosides, saponins, flavonoids, cardiac glycosides, tannins, alkaloids and triterpenes. ^[33] A report on pharmacognostic and phytochemical investigation of leaves of *Malvastrum coromandelianum* also mentioned presence of alkaloids, tannins and flavonoids along with analgesic and anti-inflammatory activities of leaf extract. ^[34]

Additionally, p-coumaric acid, beta-sitosterol and daucosterol have been reported from leaves of the plant. ^[7] Coumaric and related acids are present in whole grain cereals and have antioxidant effects, which can help relieve oxidative stress occurring in diabetes. ^[35] Beta-sitosterol possesses both antihyperglycemic and analgesic properties. ^[36, 37] Daucosterol is reportedly a strong alpha-glucosidase inhibitor and so can be beneficial in diabetes. ^[38] Thus these phytoconstituents reportedly present in *A. comosus* leaves can also account for the observed antihyperglycemic (improved glucose tolerance) and analgesic activities.

Ananas comosus is widely available in Bangladesh, and since its leaves are discarded, they can form a cheap and alternative source for lowering high blood sugar in diabetic patients and for alleviating pain, and so can be beneficial particularly for the rural people of the country.

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