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Anti-diabetic activity of *Eugenia Operculata* roxb. In streptozotocin induced diabetic mice

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ABSTRACT

The anti-diabetic effect of aqueous and ethanol extract of leaves of *Eugenia operculata* Roxb. was evaluated in streptozotocin induced diabetic mice. Streptozotocin is a synthetic nitrosoureido glucopyranose derivative isolated from fermentations of *Streptomyces acromogenes*. STZ induced diabetes by selectively destroying the insulin producing β cells by inducing necrosis. Extract (250mg/kg b.w) was administered orally to albino mice both normal and diabetic for 21 days. The anti-diabetic potential was assessed by determining oral glucose tolerance, fasting blood glucose, urine glucose, change in body weight, liver glycogen content and serum lipid profile. In oral glucose tolerance test, reduction of fasting blood glucose level was observed after 60 minutes of extract and standard administration. At the end of experiment maximum reduction of blood glucose level was seen significantly ($p<0.001$) in ethanol treated group when compared with diabetic group and ($p<0.05$) with aqueous treated at a dose of 250mg/kg b.w. The body weight of treated diabetic mice was also seen regained as compared to diabetic control. Serum lipid levels reversed towards near normal and gradual decrease of urine glucose level. The extract treatment also showed a significant increase in the liver glycogen content. The results show that leaves of *Eugenia operculata* possess significant anti-diabetic activity.

Keywords: *Eugenia operculata*, Anti-diabetic, Medicinal plant, Streptozotocin, Mice.

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INTRODUCTION

Diabetes mellitus is a complex and a multifarious group of disorders that are characterized by shortage or lack of insulin secretion or action or reduced sensitivity of the tissue to insulin [1]. resulting in elevated blood glucose levels, a condition known as hyperglycemia. Persistent hyperglycemia or high glucose levels leads to certain life threatening long term complication causing damages and failure of various organs and cells [2, 3, 4, 5, 6]. The vast majority of cases of diabetes fall into two broad categories, Type 1 and Type 2 diabetes. Type 1 accounts for only 5 to 10% of people with diabetes, it is caused by an absolute deficiency of insulin secretion. Type 2 accounts for 90 to 95% and it is caused by a combination of resistance to insulin action and an inadequate compensatory insulin secretory response [7].

Currently type 2 is the most common type of diabetes mellitus affecting more than 371 million people globally in the year 2012 comparing to 366 million in 2011 and is expected to rise to 552 million by 2030. These equals to approximately three new cases every ten seconds or almost ten million per year. Roughly 80% of people with diabetes are in developing countries, of which India and China share the larger contribution and these countries will also see the greatest increase over the next few years [8]. India is presently home to 62 million diabetics, an increase of nearly 2 million in just one year. By 2030, India's diabetes numbers are expected to cross the 100 million mark. The recently published ICMR-INDIAB national study reported that there are 62.4 million people with type-2 diabetes and 77 million people with pre diabetes in India. So diabetes burden are getting worse in India and is also ways ahead then its immediate neighbors[9].

Management of diabetes without any side effect is still a challenge for medical systems. This leads to an increasing search for improved antidiabetic drugs. Besides the therapy based on chemotherapeutic agents, the present century has progressed towards naturopathy. Thus medicinal plant plays an emerging role in treatment and management of lifelong prolonging disease, diabetes mellitus, providing a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities, especially in developing countries where resources are meager. Few of plant treatments used in traditional medicine for diabetes have received scientific scrutiny, and the World Health Organization has recommended that this area warrants attention[10].

A wide and diverge range of plant have been reported in the Indian literature that have beneficial effects in the treatment of diabetes possessing hypoglycemic properties but most claims are anecdotal and few have received adequate medical or scientific evaluation. However, only a few have been subjected to detailed scientific investigation therefore, still there is a need for modern

research in the identification of phytochemical compound(s), their target(s) and their modes of action and combination therapy of plant products with synthetic drugs that will provide treatment for all and justify the role of novel traditional medicinal plants having anti-diabetic potential..

Eugenia operculata Roxb. (E.operculata) also known as *Cleistocalyx operculata* is a small or medium sized evergreen tree widely distributed within Sub-Himalayan forests and tract. Previous reports revealed that the *E.operculataperculata* buds had various biological activities in vitro and in vivo such as anticancer, antitumor, antihyperglycemic and cardio tonic action [11,12,13,14]. The stem bark is used for ritual and religious in North-Eastern parts of India. Also in folk medicine, the ash of dried bark is given in a dose of 1.25g with water on empty stomach or 1hr before lunch and dinner for 40 days to diabetic patients. The blood glucose lowering effect of *E.operculata* extracts have been studied and confirmed using experimental animal models of diabetes. Aqueous extract of *E.operculata* flower buds inhibited the rat intestinal α glucosidase, maltase and sucrase activities, with IC_{50} values of 0.70 and 0.47mg/ml respectively, inhibition of the activity of carbohydrates hydrolyzing enzymes plays an important role in the prevention and treatment of diabetes. Again prolonged administration of *E.operculata* extract at a dosage of 500mg/kg b.w for 8 weeks to STZ induced diabetic rats clarified more antihyperglycemic effects viz restoration of the weight gain, reduction of blood glucose and urine volume [15].

However, despite the various bioactive phytochemical constituents and diverse medicinal properties attributed to this plant, no detailed biochemical studies have been carried out to shed light on the role of *E.operculata* leaves in diabetes. Hence, the present study was carried out in an attempt to investigate the possible mechanism(s) of the extract of *E.operculata* in the management of diabetes in STZ induced diabetic mice.

MATERIALS AND METHOD

Collection of plant materials:

Fresh leaves of *E.operculata* (Myrtaceae) were collected from an area around Lilong Chajing of Manipur, India in the month of April, 2010. The plant was identified and authenticated as *Eugenia operculata* Roxb., by Botanical Survey of India, Shillong and a voucher specimen was deposited at the Department of Biotechnology, Assam University.

Animal model:

Albino mice (20-35 g) of either sex were employed in this study. The mice were bought from Pasteur Institute, Shillong and were maintained under standard laboratory conditions at 25 ± 2°C,

relative humidity 50 ± 15% and normal photo period of 12hr light and 12hr dark cycle. The animal had free access to water and standard diet.

Sequential extraction of *E.operculata* leaves:

Fresh leaves of *E.operculata* leaves were first rinsed with tap water and then with distilled water for several times. Then air dried at room temperature under shade, cut into small pieces, grounded and homogenized into a fine powder using electric grinder. The finely ground air-dried leaves (50gm×4) was sequentially extracted using soxhlet apparatus with solvents of increasing polarity (petroleum ether, ethyl acetate, methanol, ethanol and water). The marc obtained after every extraction was completely dried and weighed. The residue obtained after petroleum ether extraction was dried and then extracted with ethyl acetate to obtain ethyl acetate extract. The same processes were followed for methanol, ethanol and aqueous extract also. The extracts collected from each solvent were evaporated to dryness and then lyophilized for further use.

Alpha amylase inhibitory activity:

For this assay the Starch solution (0.5% w/v) was obtained by boiling and stirring 0.25 g of potato starch in 50 ml of deionized water for 15 min. The enzyme solution (0.5 unit/ml) was prepared by mixing 0.001 g of α-amylase (EC 3.2.1.1) in 100 ml of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. The extracts polyherbal combinations were dissolved in DMSO to give concentrations from 10 to 100 mg/ml (10, 20, 40, 60, 80,100 mg/ml). The color reagent was a solution containing 96 mM 3, 5-dinitrosalicylic acid (20ml), 5.31 M sodium potassium tartarate in 2 M sodium hydroxide (8 ml) and deionized water (12 ml). 1 ml of each plant extract combinations and 1 ml enzyme solution were mixed in a tube and incubated at 25°C for 30 min. To 1 ml of this mixture was added 1 ml of starch solution and the tube incubated at 25°C for 3 min. Then, 1 ml of the color reagent was added and the closed tube placed into an 85°C water bath. After 15 min, the reaction mixture was removed from the water bath and cooled thereafter, diluted with 9 ml distilled water and the absorbance value determined at 540 nm in spectrophotometer. Individual blanks were prepared for correcting the background absorbance. In this case, the color reagent solution was added prior to the addition of starch solution and then the tube placed into the water bath. The other procedures were carried out as above. Controls were conducted in an identical fashion replacing plant extract polyherbal combinations with 1 ml DMSO. Acarbose solution (at the concentrations of 10, 20, 40, 60, 80, 100 µg/ml) was used as positive control. The inhibition percentage of α-amylase was assessed by the following formula:

% of Inhibition = [O.D Control – O.D test / O.D control] X 100

Alpha glucosidase inhibitory activity:

A volume of 50 µl of *E.operculataperculata* extracts (water, ethanol, methanol, ethyl acetate and petroleum ether) and 100 µl of 0.1 M phosphate buffer (pH 6.9) containing yeast α-glucosidase solution (1.0 U/ml) were incubated at 25°C for 10 min. After pre-incubation, 50 µl of 5mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each at 5 sec intervals. The reaction mixtures were incubated at 25°C for 5 min. After incubation, absorbance readings were recorded at 405 nm and compared to a control which had 50 µl of buffer solution in place of the extract. The α-glucosidase inhibitory activity was expressed as inhibition % and was calculated as follows:

% of Inhibition = [O.D Control – O.D test / O.D control] X 100

Induction of experimental diabetes:

Diabetes mellitus was induced in overnight fasted albino mice by intraperitoneal injection of freshly prepared STZ dissolved in cold 0.01M citrate buffer, pH 4.5 at a dosage of 45mg/kg b.w daily for five consecutive days. The blood glucose concentration was measured every week from the day of STZ injection. Administration of multiple low doses of STZ produces significant hyperglycemia from 1st week. The animals were observed to be diabetic from 2nd week. After 2nd week of STZ injection animals with blood glucose concentrations increasing by more than 40% were considered diabetic and were included in this study [16, 17].

Toxicity studies:

Acute toxicity studies were carried out on mice. Extracts were administered at doses of 100, 300, 500, 1000 and 3000mg/kb b.w to five groups of mice each containing 6 animals. After administration of extracts the animals were observed for the first 3hrs for any toxic symptoms followed by observation at regular intervals for 24hrs up to 7 days. At the end of study the animals were also observed for general morphological behavior and mortality.

Acute effect of *E.operculata* extract on blood glucose levels:

Glucose tolerance tests were performed in experimental and control mice according to the method described by [18, 19] with some modification. Briefly, two group of six normal mice and two group of six STZ induced diabetic mice were given *E.operculata* aqueous and ethanol extract at a dose of 250mg/kg b.w by gavages. Another group of normal and STZ induced diabetic mice were given standard drug glibenclamide at a dose of 5mg/kg b.w. A control group of normal mice and a control group of diabetic mice received vehicle at the same time. Grouped mice were subjected to overnight fasting from 8.00pm to 8.00am. After overnight fasting blood samples were taken from all groups at 8.01am for recording 0th min blood glucose level. One hour later, after giving

treatment glucose at the dose of 2.5mg/kg b.w were administrated to all groups. Blood glucose levels were then measured at 30, 60 and 120 minutes after glucose loading using glucometer. Blood glucose tolerance curves of experimental mice were plotted and compared with those of control groups.

Long term antidiabetic effects of *E.operculata* extracts:

Animals were divided into seven groups of six each. First group comprised of normal control and were given only gum acacia. Second to sixth group comprises of STZ induced diabetic mice. Second group received gum acacia and served as diabetic control. Third group received the standard glibenclamide at the dose of 5mg/kg b.w. Fourth and fifth group were given *E.operculata* aqueous extract and sixth and seventh group were given *E.operculata* ethanol extract, dose of 250 and 500mg/kg b.w. The treatment was continued for 21 days and fasting blood glucose level was monitored for every seven days after initiation of treatment. Mice were sacrificed at the end of the experiment, on 21st day and the blood sample were collected to determine the effect of *E.operculata* extract on various biochemical parameters.

Effects of *E.operculata* extract on lipid profile:

Blood sample were collected by the cardiac puncture method, in the centrifuge tubes and allowed o clot for 30min at room temperature. Blood sample were centrifuged at 3000rpm for 20min. Serum was separated as supernatant and stored at -20^{o}C until analysis.

Determination of triglyceride levels:

Triglycerides was estimated by GPO/PAP method using triglycerides kit. Working reagent was prepared by dissolving contents of bottle 2 enzyme (lipoprotein lipase, glycerol kinase, glycerol-3-phosphate oxidase, peroxidase, 4-aminoantipyrine and adenosine tri phosphate) into one bottle of reagent 1 buffer (3,5 dichloro-2-hydroxybenzene sulphonate, pH 7.0). It was swirled to dissolve and allowed to stand for 10 min at room temperature. Serum triglycerides were hydrolyzed to glycerol and fatty acids by lipase enzyme. In the presence of ATP and glycerol-kinase, glycerol was converted into glycerol-3-phosphate and adenosine diphosphate (ADP). Glycerol-3-PO_4 oxidase dissociates glycerol-3-phosphate into dihydroxy-acetone phosphate and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with 4-aminoantipyrine to form a coloured complex. The intensity of the colour developed was proportional to the triglycerides concentration and was measured photometrically at 505 nm. The instrument was adjusted to zero with distilled water. 10µl sample was taken with the help of pipette into a cuvette. It was mixed and incubated for 5 m. at 37°C. The absorbance of the samples and standard of 200 mg/dl

concentration was read out against the blank. The colour should be stable for 30 m. Triglyceride was estimated by using the following formula:

Triglyceride (mg/dl) =Absorbance of Samples/Absorbance of standard x Conc. of standard (mg/dl)

Estimation of total serum cholesterol:

Total serum cholesterol was estimated by enzymatic method (Autopak, Siemens Ltd). Working reagent was prepared by dissolving contents of one bottle of reagent 1 enzymes (cholesterol esterase, cholesterol oxidase, peroxidase, 4-aminoantipyrine) into one bottle of reagent 1A buffer (pipes buffer, phenol, sodium cholate, pH 6.95). It was swirled to dissolve and allowed to stand for 5min at room temperature. Serum cholesterol ester was hydrolyzed to cholesterol and fatty acid by cholesterol esterase. Cholesterol oxidase converted cholesterol into cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, hydrogen peroxide reacts with 4-aminoantipyrine to form a coloured complex. The intensity of the colour developed was propotional to the concentration of cholesterol in the sample and was measured photometrically at 500nm. The instrument was adjusted to zero with distilled water. 10µl each of serum as a sample and standard sample along with 1ml reaction reagent were pipette into a cuvette using a micropipette.

Estimation of serum lipid:

Phosphotungstate method was used to estimate the serum lipids like very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol level. The clear supernatant after removal of VLDL and LDL, containing HDL was used for determination of HDL-Cholesterol (HDLc). The VLDL and LDL from serum were precipitated by phosphotungstate in the presence of magnesium ions.

Serum/Plasma Phosphotungustate, mg HDL+(LDL+VLDL+Chylomicrons)

VLDL-Cholesterol (VLDLc) and LDL-Cholesterol (LDLc) were respectively calculated by using Frederickson- Friedwald's formula as follows:

LDL Cholesterol = Total Cholesterol – Trigyceride/5- HDL Cholesterol.

VDDL Cholesterol= Triglyceride/5

For this 0.5 ml of serum was taken in a test tube and 0.5 ml of precipitation reagent was added. The mixture was shaken thoroughly and left to stand for 10 m at 25 to 30°C and then centrifuged for 20 m at 4000 rpm. Within 2 hrs after centrifugation, the clear supernatant was used for the determination of HDL-Cholesterol. The supernatant containing 0.05 ml was taken in a test tube and 1 ml reaction solution was added to it. In another test tube, 0.1 ml distilled water was taken

and 1 ml reaction solution was added. The mixtures were mixed thoroughly, incubated for 5 min at 37°c and measured for the absorbance of the sample against blank reagent at 510 nm.

Liver glycogen estimation:

The determination of glycogen in liver was done by anthrone reagent method with slight modification [20]. Purified anthrone (500 mg), thiourea (10 g) and 1 liter of 72 % sulfuric acid were placed in a flask. The mixture was heated up to 80-90°c and then cooled and stored in a refrigerator. Stock solution of standard was prepared by dissolving 100 mg of dry glucose in 100 ml of saturated benzoic acid solution. 5 ml stock solution was placed in a 100 ml volumetric flask and the volume was made up with saturated benzoic acid solution. Liver was blended by blender under trichloroacetic acid (TCA) and homogenized for 3 min. The homogenate was poured into a centrifuge tube. The supernatant fluid was centrifuged and decanted upon an acid-washed filter paper placed in a funnel and drained into a graduated cylinder. The residue was quantitatively transferred to the blender with TCA and homogenized again for 1 m. The mixture was centrifuged and the supernatant fluid was poured through the same filter. Two more extractions were made in the same manner. The desired volume was made up with 5% TCA and the solution was mixed thoroughly. 1 ml of the TCA filtrate was pipette into a 15 ml Pyrex centrifuge tube. Duplicate samples of each unknown were analyzed to obtain the most reliable results. To each tube, 5 volumes of 95 percent ethanol were added with careful blowing. This was checked by noting the absence of an interface. The tubes were capped with clean rubber stoppers and allowed to stand overnight at room temperature. After precipitation was completed, the tubes were centrifuged at 3000 r.p.m. for 15 m. The clear liquid was gently decanted from the packed glycogen and the tubes were allowed to drain in an inverted position for 10 m. The glycogen was dissolved by adding 2 ml of distilled water. Blank reagent was prepared by pipetting 2 ml of water into a clean centrifuge tube. A standard was prepared by pipetting 2 ml of standard glucose solution, containing 0.1 mg of glucose, into a similar tube. At this point 10 ml of anthrone reagent was delivered into the centre of the each tube with vigorous, but consistent, blowing to ensure good mixing. As each tube received anthrone reagent, it was tightly capped with an air condenser and placed in a cold tap water bath. After the temperature of all tubes had reached the temperature of cold water, they were immersed in a boiling water bath to a depth a little above the level of the liquid in the tubes for 15 m and then removed from water bath and cooled to room temperature. The absorbance was read at 620 nm after adjusting the calorimeter with the blank reagent. The calculation of glycogen content was done by using the following formula

DU/DS x 0.1 x Volume of Extract/100gm. of Tissue x 100 x 0.9 = mg. of glycogen per 100gm. of tissue.

RESULTS AND DISCUSSION:

One hundred grams of fine powder was extracted in Soxhlet extractor (40 to 45^0C for 12 hours) with 500ml of petroleum ether. The extracted solution collected at the bottom of round flask was evaporated by means of rotary evaporator to yield 5.4gm of extract. The residue obtained from filtration of petroleum ether extract was dried and then extracted with acetone for 4 hrs; the extracted solution was then discarded. The residue collected was dried and then extracted with 500ml of ethyl acetate. The extracted solution was collected and dried by means of rotary evaporator to yield 0.8gm of extract. The residue obtained from filtration of ethyl acetate extract was then dried and again extracted with 500 ml of ethanol; the extracted solution was collected and dried to yield 24.4 gm of extract. The residue obtained was further dried and then extracted with 500 ml of methanol; the yield obtained from methanol extract was 8.4gm. Again, residue left was dried and extracted with distilled water; the yield obtained from aqueous extract was 23.54gm.

Alpha amylase inhibition:

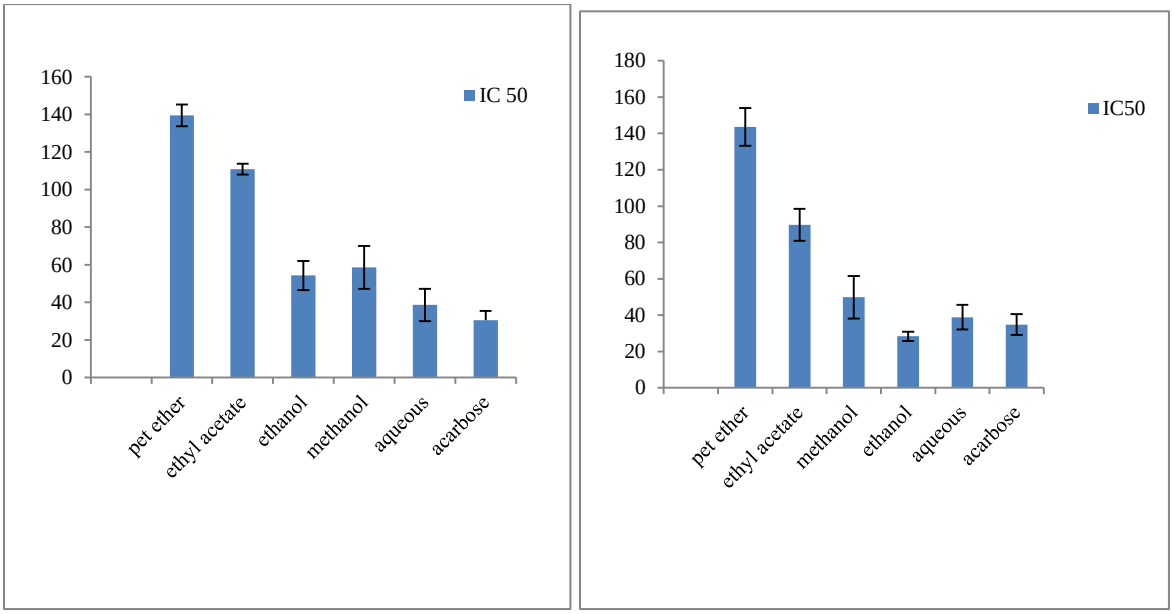
High intake of carbohydrate and sucrose rich food is one of the main causes of no-insulin diabetes mellitus. Potent inhibitors of mammalian α-amylase found in medicinal plants involved in digestion of carbohydrates can significantly decrease the post prandial increase of blood glucose. In vitro α-amylase inhibitory studies demonstrated that all crude *E.operculata* leaves extracts (petroleum ether, ethyl acetate, ethanol, methanol and aqueous) screened for α-amylase inhibitory activity inhibited porcine pancreatic alpha amylase. The crude ethanol and aqueous extract shows maximum inhibitory activity against all the extracts with 96.39 and 92.62 percent inhibition respectively. Petroleum ether extract shows the minimum inhibitory activity with 33.62% inhibition.

The concentration of the extracts required for 50% inhibition (IC_{50}) for all the extracts were determined from corresponding dose response curves of percentage inhibition versus inhibitor concentration and compared with those of acarbose, a known inhibitor of α-amylase. Ethanol extract of *E.operculata* leaves appeared to be better inhibitors of porcine pancreatic α-amylase than acarbose with IC_{50} value, 28.32µg/ml compared with 34.83µg/ml as shown in Figure 1(A). In the current study, the mode of inhibition of *E.operculata* ethanol extract was found to be competitive suggesting that some of the α – amylase inhibitory components in extract may be structural analogs of the substrate of α-amylase or may be due to the changes of the conformation of

carbohydrate binding regions of α – amylase, that catalyze hydrolysis of the internal α-1,4 glucosidic linkages in starch and other related polysaccharides[21, 22].

Alpha glucosidase inhibition:

Inhibition of the activity of carbohydrate-hydrolyzing enzymes plays an important role in the prevention and treatment of diabetes. The αglucosidase inhibiting activity of *E.operculataperculata* extract was determined by measuring the release of P-nitrophenol from P-nitrophenyl–α-D-glucopyranose. *E.operculata* leave extracts (petroleum ether, ethyl acetate, ethanol, methanol and water) investigated in the current study demonstrated dose dependent yeast α-glucosidase inhibitory activity. This observation suggests that yeast α-gucosidase is inhibited by both polar and nonpolar components of the leave extract of *E.operculata*. Of all the extract tested, aqueous extract showed highest inhibition activity with IC50 38.61µg/ml followed by ethanol extract, IC50 54.25 µg/ml but lesser than the reference drug acarbose with IC50 30.57µg/ml as shown in Fig 1(B). Previous studies also showed inhibitory activity of *E.operculata* bud extract against the α-glucosidase, rat-intestinal maltase and sucrase activities. Thereby considering *E.operculata* as a promising material for preventing and treating diabetes.



(A) (B)

Figure 1: IC_{50} values of *E.operculata* crude extracts and acarbose against (A) procine pancreatic alpha amylase and (B) yeast alpha glucosidase.

Acute oral toxicity test:

To establish the safety of the extracts, a dose dependent ethanol and aqueous extract up to 3000mg/kg b.w were administered to normal mice of both sexes. No significant toxic signs or

death of animal was observed during the 7 days of observation. None of the animal showed clinical toxic signs such as anorexia, depression, lethargy, convulsion, ataxia, and diarrhea and also, no mortality happened throughout the examination.

Effect of *E.operculataperculata* ethanol and aqueous extract on oral glucose tolerance test: Figure 2 and 3 shows the effect of *E.operculata* ethanol and aqueous extract on postprandial blood glucose levels in normal and STZ induced diabetic mice when fed simultaneously with glucose (2.5mg/kg b.w). The blood glucose levels of the entire group increased sharply 30 minutes after an oral administration of glucose and then decreased steadily thereafter. In case of normal untreated mice rise in blood glucose level falls from 66.68 % after 30 minutes to 27.48 % at 120 minutes. Whereas in case of diabetic control group there is no significant difference in blood glucose level. In extract treated groups of both normal and diabetic mice, there is a strong and significant opposing effect on the rise of serum glucose level when fed simultaneously with glucose. After 120 minutes AqTNM, ETNM, AqTDM, ETDM shows an inhibition of 72.9%, 76.06%, 86.30%, and 88.65% from 40.75%, 42.03%, 81.07%, &78.50 % respectively. The suppression of postprandial rise in normal mice by *E.operculata* ethanol extract was almost similar to that caused by glibenclamide, whereas the suppression in diabetic mice was significantly lower than that caused by standard.

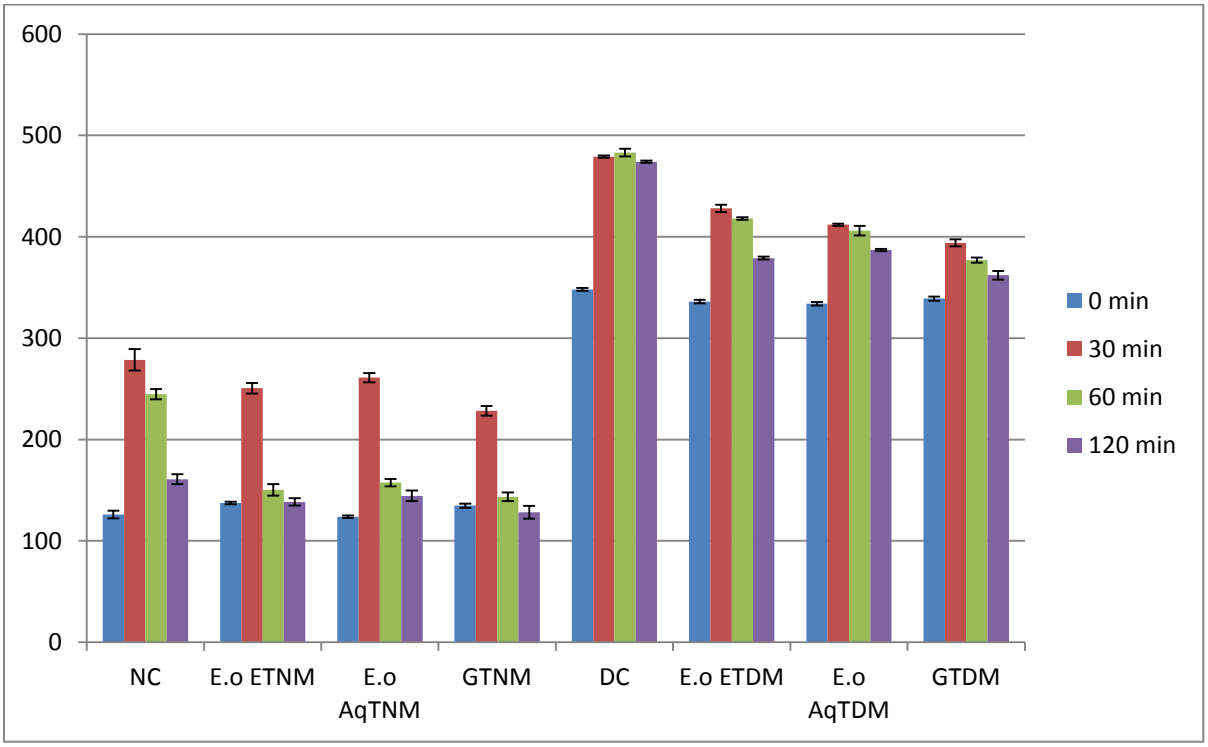
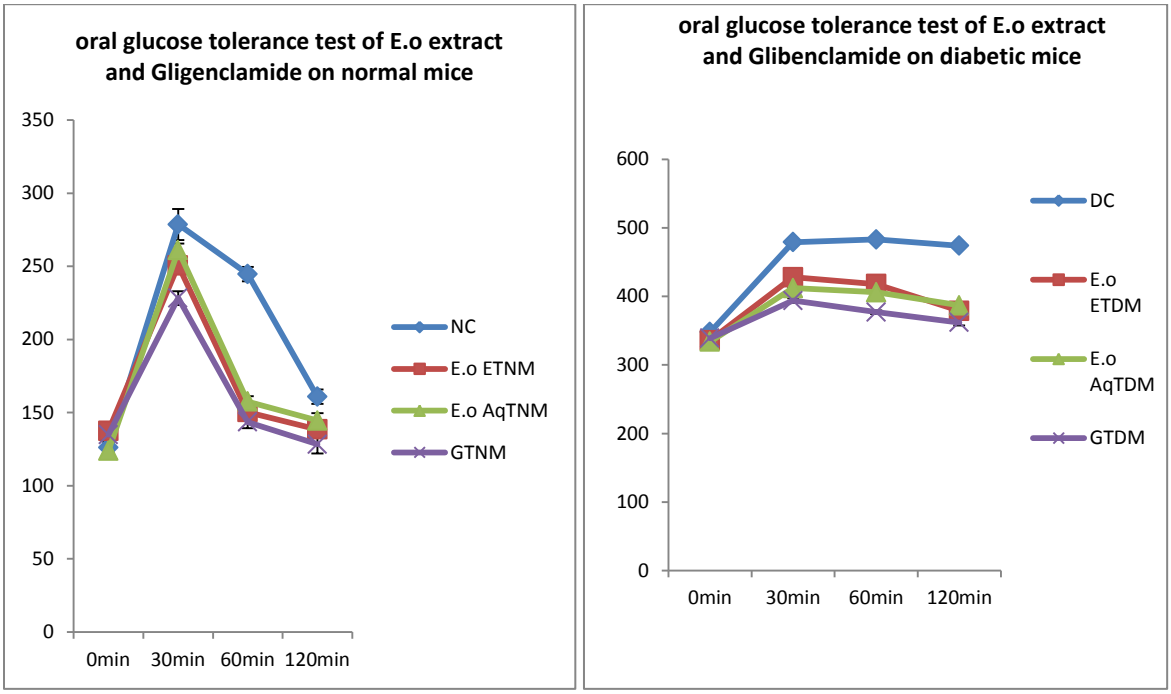


Figure 2: Effect of *E.operculata* extract on oral glucose tolerance test in normal and diabetic mice



(A) (B)

Figure 3: Effect of *E.operculata* extracton percentage inhibition of glucose rise (A=treatment on normal mice; B= treatment on diabetic mice)

NC=Normal control, DC= Diabetic control, *E.operculata*ETNM= *E.operculata* ethanol treated normal mice, *E.operculata*AqTNM= *E.operculata* aqueous treated normal mice, GTNM= Glibenclamide treated normal mice, *E.operculata*ETDM= *E.operculata* ethanol treated diabetic mice, *E.operculata*AqTDM= *E.operculata* aqueous treated diabetic mice, GTDM= Glibenclamide treated diabetic mice

Effects on fasting blood glucose level:

Induction of diabetes in the experimental mice was confirmed by the presence of high blood glucose level. In this study, the level of glucose for diabetic control group, group 2 was significantly ($P<0.05$) increased from 296.66±15.1 from day 1 to 548.50±19.73 on 21st day. Glibenclamide treated diabetic mice of standard group, group 7 showed significant reductions ($p<0.001$) in blood glucose level starting from first week after the initiation of treatment to the end of the experiment i.e from 167.83±10.88 to 113.33±1.54. Administration of *E.operculata* aqueous and ethanol at dosages of 250 and 500mg/kg b.w to diabetic mice tends to bring down the values of glucose level to near normal. Among these two doses of both extract, the dose of 250mg/kg b.w showed better result with a fall of glucose level from 308.66±27.78 to 157.33±17.14 in aqueous extract and 444.00±17 to 204.33±19.75 in ethanol extract from the starting of treatment to the end of experiment. Whereas group treated withETDM and AqTDM 250mg/kg b.w groups were more

significant (p<0.001) than GTDM group. Therefore the dose of 250mg/kg b.w of *E.*Aq and *E.* E were selected for further analysis.

Table 1: Effect of *E.operculata* extract in both normal diabetic mice. ***P<0.001,p<0.01 as compared to diabetic control, a*p<0.05 when compared to AqTDM2500mg/kg b.w., b***p<.001 when compared to GTDM.**

Groups		Variables blood glucose level before treatment	After 1st week	After 2nd week	after3rd week
normal control	mean± Std. Error of Mean	135.1667 3.15612	137.5000 2.47319	140.5000 7.42406	109.8333 6.95422
diabetic control	mean± Std. Error of Mean	296.6667 15.12099	454.8333 32.37326	516.8333 26.35706	548.5000 19.73787
AqTDM 250mg/kg b.w	mean± Std. Error of Mean	308.6600 27.78209	163.16** 16.76786	160.0*** 15.56706	157.33***b 17.14189
AqTDM 500mg/kg b.w	mean± Std. Error of Mean	325.5000 15.39426	181.16*** 16.34098	130.83*** 8.81823	127.5** 6.47431
ETDM 250mg/kg b.w	mean± Std. Error of Mean	440.0000 17.63519	338.5***a***b*** 10.99924	189.66***b 20.73269	204.33***a*b*** 19.75292
ETDM 500mg/kg b.w	mean± Std. Error of Mean	360.1667 22.96144	136.83*** 20.12530	126.16** 19.55406	126.33** 11.22398
GTDM	mean± Std. Error of Mean	363.3333 11.63806	167.83*** 10.88858	130.83*** 7.48072	113.33***a* 1.54200

Effects on urine glucose:

The urine sugar levels in normal and diabetic group of mice are mentioned in Table 2. The normal control mice showed absence of sugar in urine. The urine sugar levels of different groups of diabetic animals treated with standard drug, glibenclamide and *E.operculata*Aq and *E.operculata*E for 21days decreased towards the normal level. Whereas, in case of diabetic control group the urine sugar level keeps on rising consistently.

Table 2: Effect of *E.operculata* extract and glibenclamide on urine glucose level. Keys (+)=mild, (++)= moderate, (+++)=higher, (++++)=severe.

Group	Before treatment	After 1st week	After 2nd week	After 3rd week
Normal control	Nil	Nil	Nil	Nil
Diabetic control	+++	+++	++++	++++
AqTDM 250mg/kg b.w	+++	++	++	+
AqTDM 500mg/kg b.w	+++	++	++	+
ETDM 250mg/kg b.w	+++	++	+	+
ETDM 500mg/kg b.w	+++	++	++	+
GTDM	+++	++	++	+

Effects on body weight:

Induction of diabetes with STZ is associated with a characteristic loss of body weight, which is probably due to muscle wasting [23]. In our study there was a significant weight loss in the vehicle treated diabetic mice, this reduction was found to be statistically significant ($P<0.05$) when compared with normal control group. These reduced body weight were found to increase significantly ($p<0.001$) upto 28.86±0.4 and 29.50±0.5 from 27.83±0.6 and 29.09±0.3 for group treated with Aq250mg/kg b.w and E500mg/kg b.w respectively. *E.operculata* extract of leaves showed improvement in their body weight, indicating that the aqueous extract had beneficial effect in preventing loss of body weight of diabetic mice. The probable mechanism of this benefit is due to its effect in controlling muscle wasting i.e.by reversal of antagonism [24]. The metabolic disturbances were corrected after the plant extract was administered for 21 days as shown by a reduction in polyphagia, polyurea and polydipsia in diabetic mice treated with plant extract.

Table 3: Effect of *E.operculata* on body weight (gm). *p<0.05,p<0.01***p<0.001 compared to diabetic control.**

Groups		body weight before treatment	body weight after 1 week	body weight after 2nd week	body weight after 3rd week
normal control	Mean	27.3533	27.1133	26.9967	27.6033
	Std. Error of Mean	84545	72842	58750	70054
diabetic control	Mean	29.6683	28.4017	27.9083	24.3783
	Std. Error of Mean	1.10640	1.04457	95070	48098
AqTDM 250mg/kg b.w	Mean	27.8333	26.9367	25.8150	28.86***
	Std. Error of Mean	60093	60019	55176	40361
AqTDM 500mg/kg b.w	Mean	26.0050	24.86**	26.1917	26.96*
	Std. Error	59654	.55727	56739	1.00007

	of Mean				
ETDM	Mean	24.9750	25.30*	25.44*	25.3433
250mg/kg b.w	Std. Error of Mean	.31860	.43872	.41398	.19525
ETDM	Mean	29.0950	28.8250	28.9600	29.50***
500mg/kg b.w	Std. Error of Mean	.30119	.30692	.24209	.21472
GTDM	Mean	27.1600	26.9567	26.9167	25.7367
	Std. Error of Mean	.25198	.33580	.21464	.27523

Effect on serum lipid profile:

Hyperglycemia produced by STZ exhibited marked increase in serum triglycerides and total cholesterol. Under normal conditions the enzyme lipoprotein lipase hydrolyses triglycerides. Diabetes mellitus results in failure to activate this enzyme thereby causing hypertriglyceridemic. Elevated serum total cholesterol, triglycerides and decreased high density lipoprotein level were observed in diabetic control mice. In contrary diabetic mice treated with *E.operculata* extract at a dose of 250 mg/kg for 21 days significantly ($p<0.01$) reduced total cholesterol, triglycerides, low density lipoprotein and very low density lipoprotein associated with significant increase in HDL and the same has been obtained from glibenclamide treated group. Normalization of serum lipid profile by *E.operculata* extract and glibenclamide might be due to stimulation of insulin secretion from beta cells or may be due to low activity of cholesterol biosynthesis enzymes and or low level of lipolysis, which are under the control of insulin [25]. The observed hypolipidaemia effect may be because of decreased cholesterogenesis and fatty acid synthesis. Significant lowering of total cholesterol and elevation of HDL cholesterol are very desirable biochemical states for prevention of atherosclerosis and ischemic conditions[26]. There are reports that other medicinal plants have hypoglycemic and hypolipidemic effects that could prevent or be helpful in reducing the complications of lipid profile seen in some cases of diabetes in which hyperglycemia and hypercholesterolemia coexist. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids [27]. Further it has been reported that diabetic rats treated with insulin shows normalized lipid levels [28]. Thus, the results indicate that *E.operculata* also may possess insulin like action by virtue of the ability to lower the lipid levels. These results are similar to earlier reports observed with the other plant [29].

Table 4: Effect of *E.operculata* on lipid profile. ***p<0.001, **p<0.01 compared with diabetic mice.

Groups		STG	STC	HDLc	LDLc	VLDLc
normal control	Mean ±	92.16	81.00	37.16	25.40	18.43
	Std. Error of Mean	0.47	0.44	0.60	0.33	0.09
diabetic control	Mean ±	133.33	156.00	16.83	112.50	26.66
	Std. Error of Mean	0.80	0.63	0.60	0.26	0.16
AQTDM250mg/kg b.w	Mean ±	114.50**	130.00**	31.16**	75.93**	22.90**
	Std. Error of Mean	0.71	1.52	0.65	1.00	0.14
ETDM250mg/kg b.w	Mean ±	119.00***	134.50**	24.16**	86.53**	23.80**
	Std. Error of Mean	.00	1.89	0.87	1.38	.00
GTDM	Mean ±	102.16***	112.83***	25.33**	67.06**	20.43**
	Std. Error of Mean	1.88	2.28	1.56	1.35	.37

Effect on liver glycogen content:

Glycogen, the preliminary intracellular storable form of glucose in various tissues is a direct reflection of insulin activity, as insulin promotes its deposition by stimulating glycogen synthetase and inhibiting glycogen phosphorylase. The observed reduction of liver glycogen level in diabetic mice was consistent with the earlier result indicating that it was possibly due to lack of insulin that results in the inactivation of glycogen synthase enzyme [36]. As, STZ caused selective destruction of beta cells of the islet of langerhans resulting in a marked decrease of insulin level. Furthermore, earlier studies also showed that the reduced hepatic glycogen content was normalized by insulin treatment [30]. In this study, there was a significant($p<0.001$) increase in liver glycogen level on day 21in all the treated group, AqTDM, ETDM and GTDM as compared to diabetic control group.

The significant increase in the glycogen content of the treated groups may be because of reactivation of the glycogen synthase enzyme thus confirming its insulin potentiating effect to a certain level.

Table 5: Effect of *E.operculata* extract on liver glycogen content in STZ induced diabetic mice. ***p<0.001 compared with diabetic control.

Groups	Liver glycogen content(Mean±SEM)
NC	523.66±2.85
DC	225.66±5.52
AQTDM250mg/kg b.w	373.16±12.78***
ETDM250mg/kg b.w	462.00±6.83***
GTDM	476.83±6.90***

CONCLUSION:

It is thus concluded that *E.operculata* has promising antidiabetic effect, which potentially improved abnormalities of diabetic conditions in streptozotocin induced diabetic mice. The

probable hypoglycemic effect of *E.operculata* may be due to inhibition of the activity of carbohydrate-hydrolyzing enzymes or normalization of the pancreatic cells thereby increasing serum and pancreatic insulin level. The use of this plant for diabetes is promising but the precise substances, site(s) and mechanism(s) of its pharmacological effect are still to be determined.

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