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ANTIDIABETIC AND HYPOLIPIDEMIC EFFECTS OF THE DIFFERENT FRACTIONS OF METHANOLIC EXTRACTS OF *ENTADA PHASEOLOIDES* (L.) MERR. IN ALLOXAN INDUCED DIABETIC MICE

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ABSTRACT

The antidiabetic effects of Ethyl acetate (Et Ac), Petroleum-ether (Pet ether), and Chloroform fractions from methanolic extract of the seeds of *Entada phaseoloides* were investigated in alloxan induced diabetic mice (AIDM). The effect of these fractions (200 mg/kg body weight i.p) was observed on fasting blood glucose (FBG) level and active fraction was further investigated for its dose dependent activity (250 and 350 mg/kg b. w.) on fasting blood glucose level and also on total cholesterol (TC), triglyceride (TG), serum glutamate oxaloacetate transaminases (SGOT) and serum glutamate pyruvate transaminases (SGPT) level in AIDM and found significant effects. The most significant reduction of FBG level of around 72.02% was observed for Et-Ac fraction in AIDM. A significant reduction (*p< 0.05) in serum TC and TG level of 53.00% and 57.25% respectively was also found for Et-Ac fraction of *E. phaseoloides*. The hypoglycemic and hypolipidemic activities were comparable to metformin HCl (150 mg/kg). In diabetic mice, SGOT and SGPT levels were significantly elevated that were further reduced after intraperitoneal administration of this fraction. These results indicate that Et-Ac fraction of *E. phaseoloides* have favorable effects in bringing down the severity of diabetes together with hepatoprotectivity.

INTRODUCTION: Diabetes is a chronic metabolic disorder that is characterized by either the insufficient production or the lack of response to a key regulatory hormone of the body's metabolism, insulin. Diabetes is divided into two major categories: Type 1 diabetes (insulin-dependent diabetes mellitus or IDDM) and type 2 diabetes (non-insulin dependent diabetes mellitus or NIDDM). Type- 1 diabetes, the cause is an absolute deficiency of insulin secretion and the cause of type- 2 diabetes is a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response [1]. The overall prevalence of diabetes is approximately 10 percent of the population, of which 90 percent is type 2. There are estimated 246 million people worldwide sufferings from diabetes [2]. In the United States, diabetes is the sixth leading cause of death [3]. It is predicted that by 2030, India, China and the United States will have the largest number of people with diabetes [4].

The increasing worldwide incidence of diabetes mellitus in adults constitutes a significant impact on the health, quality of life, and life expectancy of patients, as well as on the global public health care system. The long term manifestation of this disease can result in the development of vascular disorders such as retinopathy, nephropathy, neuropathy, and angiopathy [5]. Sedentary lifestyle, degree of obesity, changes in food consumption, ageing, and other concomitant medical conditions has been implicated in

this increasing prevalence in the past two decades. The present treatment of diabetes is focused on controlling and lowering blood glucose to a normal level. In conventional therapy, type 1 diabetes is treated with exogenous insulin and type 2 with oral hypoglycemic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors, and glinides, which are used as monotherapy or in combination to achieve better glycemic regulation.

Many of these oral antidiabetic agents have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge [6, 7]. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of investigation. Traditional medicines from readily available medicinal plants offer great potential for the discovery of new antidiabetic drugs. The hypoglycemic effect of several plants used as antidiabetic remedies has been confirmed, and the mechanisms of hypoglycemic activity of these plants are being studied.

Biguanides include the drug metformin, which was originally derived from a medicinal plant, *Galega officinalis*. Metformin reduces plasma glucose via inhibition of hepatic glucose production and increase of muscle glucose uptake. It also reduces plasma triglyceride and LDL-cholesterol levels [8]. The effect of *Tinospora cordifolia* W. (Menispermaceae) roots at 2.5 and 5.0 g/kg b.w. was better than that of glibenclamide [9]. Three new isoquinoline alkaloids, schulzeines A, B, and C, were isolated from the marine sponge *Penares schulzei* inhibit α-glucosidase.

The natural sweetener stevioside, which is found in the plant *Stevia rebaudiana* Bertoni (Asteraceae), has been used in the treatment of diabetes for many years in many parts of the world. Stevioside, with a mechanism for stimulating insulin secretion via direct action on the beta cells of pancreatic islets, is considered to have the potential of becoming a new antidiabetic drug for use in treatment of type 2 diabetes [10, 11].

Entada phaseoloides (L) Merr. (Fabaceae), commonly known as gilla, is a large woody vine that climbs high into the lowland forest canopy, naturally found in lowland coastal forests of Africa, Australia, Asia and the Western Pacific. Medicinally used for rheumatic lumbar and leg pains, jaundice, edema due to malnutrition, abdominal pains and colic, counterirritant, hair growth stimulant, skin itches, emetic, antidiabetic and remedy for cerebral hemorrhage. The seeds claimed to possess anti-tumor [12], genotoxicity [13], and anti-inflammatory [14]. But, antidiabetic activity is yet to be investigated and the objective of the present work was to evaluate ethno pharmacological antidiabetic activity of *Entada phaseoloides* as well as hypolipidemic activities in alloxan induced diabetic mice.

MATERIALS AND METHODS:

Plant Materials: The dried fruit of *E. phaseoloides* (locally named 'Gilla') were collected from Bandarban hill tracts, Chittagong, Bangladesh. After removing seed coat they were dried completely under the mild sun and grinded to a coarse powder and then used for cold extraction. The authenticity of the *E. phaseoloides* was identified by Mr. Md. Boctiar Uddin, Associate Professor, Department of Botany, University of Chittagong. A voucher specimen, collection # 60, dated 25/08/20010 has been kept in the Department of Botany, University of Chittagong, Bangladesh.

Preparation and Fractionation of Crude Extracts: The coarse powder was submerged in methanol (96%) and allowed to stand for several days (7-10) with occasional shaking and stirring. When the solvent become concentrated, the liquid alcohol content was filtered through cotton and then through filter paper (Whatman filter paper #1). Then the solvents were allowed to evaporate using rotary evaporator at temperature 40-45°C. Thus the highly concentrated crude extracts were obtained. They were then fractionated using Et Ac, Pet ether, and $CHCl_3$. The dried fractionated extracts were then preserved in the refrigerator for the experimental use.

Drugs and Chemicals: The active drug, Metformin hydrochloride was the generous gift samples from Square Pharmaceuticals Ltd., Pabna Bangladesh. TC and TG wet reagent diagnostic kits were the products of Cresent diagnostic kits. Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. SGPT and SGOT wet reagent diagnostic kits were purchased from AMP Medizintechnik GmbH; Austria.

Experimental Design: In the experiment, a total of 30 male Swiss Albino mice, 20-25gm; age 6-8 weeks (25 diabetic surviving mice, 5 normal mice) were used. Normal control group received only vehicle (DMSO). Diabetic control group does not receive either metformin, or plant extracts. Next one stands for metformin control group in which metformin was administered intraperitoneally at a dose of 150 mg/ kg body weight.

Et Ac, Pet ether and chloroform partitionates of *E. phaseoloides* seed extracts were administered accordingly to their respective groups intraperitoneally at the dose of 200 mg/kg body weight. The blood samples were analyzed for blood glucose content at 0, 2, 4, 8, 16, and 24 hours, respectively by using glucometer (Bioland-423, Germany).

Collection of Serum and determination of Serum TC, TG, SGOT and SGPT: After completing blood glucose level estimation mice were sacrificed and about 3-5 ml of blood was collected directly from heart by syringes, centrifuged at 4000 rpm for 10 minutes and the serum was preserved to examine, TC, TG, SGOT and SGPT concentrations by UV spectrophotometric method (Shimadzu UV-1200, Tokyo, Japan), using wet reagent diagnostic kits according to manufacturer's protocol.

Phytochemical Screening Methods: Phytochemical tests have been performed according to the literature by Nayak and Pereira[15]:

I. Test for saponins: 300 mg of extract was boiled with 5 ml water for two minutes. The mixture was cooled and mixed vigorously and left for three minutes. The formation of frothing indicated the presence of saponins.

II. Test for tannins: To an aliquot of the extract, sodium chloride is added to make to 2% strength. Then it is filtered and mixed with 1% gelatin solution. Precipitation indicated the presence of tannins.

III. Test for Triterpenes: 300 mg of extract was mixed with 5 ml chloroform and warmed for 30 minutes. The chloroform solution was then treated with a small volume of concentrated sulphuric acid and mixed properly. The appearance of red color indicated the presence of triterpenes.

IV. Test for alkaloids: 300 mg of extract was digested with 2 M HCl. Acidic filtrate was mixed with amyl alcohol at room temperature, and examined the alcoholic layer for the pink color which indicated the presence of alkaloids.

V. Test for flavonoids: The presence of flavonoids was determined using 1% aluminium chloride solution in methanol, concentrated HCl, magnesium turnings, and potassium hydroxide solution.

VI. Test for glycosides: A small amount of alcoholic extract of sample was dissolved in 1.0 ml of water and then aqueous solution of sodium hydroxide was added. Formation of a yellow color indicates the presence of glycosides.

Statistical Analysis: Data were expressed as mean ± standard error of mean (SEM). Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Scheffe's post-hoc test or students paired or unpaired t-test where appropriate. Results were considered to be significant when p values were less than 0.05 ($p<0.05$). Statistical calculations and the graphs were prepared using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com).

RESULTS: The effect of the different fractions of *E. phaseoloides* on the fasting blood glucose (FBG) level and evaluation of effect of active fraction on serum total cholesterol (TC), serum triglyceride (TG), SGOT, and SGPT levels in liver were investigated in the control and alloxan-induced diabetic mice using metformin HCl as standard antidiabetic agent.

Effect of different fractions of *E. phaseoloides* on FBG level in Diabetic Mice: The mean blood glucose concentration of control and different fractions of *E. phaseoloides*-treated animals were estimated on the 2, 4, 8, 16, and 24 hours, respectively as shown in **table 1**. Their baseline glucose concentration was also measured. Et Ac fraction of *E. phaseoloides* reduced blood glucose level to 70.97%, 48.92%, 34.27%, 45.88% and 70.67% at 2, 4, 8, 16 and 24 hours, respectively. Maximum reduction of blood glucose level of 65.72% was observed on 8 hour during the 24 hour experimental period. $CHCl_3$ fraction of *E. phaseoloides* showed reduction of blood glucose level to 76.39%, 61.89%, 50.49%, 79.87% and 90.65% at 2, 4, 8, 16 and

24 hours, respectively. Maximum reduction of blood glucose level of 49.50% was also observed on 8 hour during the 24 hour experimental period. Pet-ether fraction of *E. phaseoloides* reduced blood glucose level to 88.10%, 61.15%, 48.68%, 79.67% and 95.81% at 2, 4, 8, 16 and 24 hours, respectively. Here Maximum reduction of blood glucose level of 51.32% was also observed on 8 hour of the experiment. In case of alloxan induced diabetic mice, metformin reduced blood glucose level to 57.68%, 46.32%, 23.44%, 18.37% and 27.75% at 2, 4, 8, 16 and 24 hours, respectively. So metformin caused maximum reduction of blood glucose level of 81.63% on 16 hour of the experiment.

TABLE 1: EFFECT OF DIFFERENT FRACTIONS OF *E. PHASEOLOIDES* ON THE FBG LEVEL ON DIABETIC MICE COMPARED TO NORMAL MICE

Treatment	Blood Glucose Level (mmol/L)					
	0 hr	2 hr	4 hr	8 hr	16 hr	24 hr
Normal Control	6.16±0.88	5.34±0.29	5.34±0.56	5.60±0.44	5.74±0.58	5.12±0.42
Diabetic (D) Control	23.29±0.52	22.69±1.19	23.59±0.35	23.15±0.58	22.10±1.04	22.87±0.62
D + Metformin	29.94±0.81	17.27±0.41	13.87±0.80	7.02±0.22*	5.50±0.49*	8.31±0.55*
D + $CHCl_3$-EPE	32.20±1.04	24.60±1.18	19.93±1.11	16.26±0.54	25.72±0.83	29.19±0.63
D + Et Ac-EPE	29.29±1.16	20.79±1.13	14.33±0.52*	10.04±0.60*	13.44±0.61*	20.70±1.19
D+Pet-E- EPE	27.75±0.89	24.45±0.84	16.97±1.00	13.51±1.16*	22.11±1.18	26.59±0.86

* indicates significant changes in FBG level in diabetic mice after treatment (p< 0.05). The results are expressed as mean ± SEM. 5 mice in each group. EPE=*Entada phaseoloides* extract

Again, after dose dependent treatment of diabetic mice with Et Ac fraction of *E. phaseoloides* maximum reduction of 69.90% and 72.02% blood glucose level was observed at the dose of 250 and 350 mg/kg body weight whereas metformin showed maximum reduction of 81.62% blood glucose level on 16 hour of 24 hour treatment period shown in **table 2**.

TABLE 2: BLOOD GLUCOSE (FBG) LEVEL AFTER TREATMENT OF METFORMIN AND ETHYL ACETATE FRACTION OF *ENTADA PHASEOLOIDES* AT DIFFERENT DOSES ON ALLOXAN-INDUCED DIABETIC MICE

Treatment	Blood Glucose Level (mmol/L)					
	0 hr	2 hr	4 hr	6 hr	16 hr	24 hr
Normal Control	6.16±0.88	5.12±0.29	5.74±0.56	5.60±0.44	5.34±0.58	5.34±0.42
Diabetic (D) Control	23.29±0.52	22.69±1.19	23.59±0.35	23.15±0.58	22.10±1.04	22.87±0.62
D + Metformin	29.94±0.81	17.27±0.41	13.87±0.80	7.02±0.22*	5.50±0.49*	8.31±0.55*
D + Et Ac-EPE (250 mg/kg)	27.81±0.35	19.11±0.52	13.08±0.55	9.03±0.68*	8.37±0.91*	11.90±1.19
D + Et Ac-EPE (350 mg/kg)	26.38±1.05	16.72±0.65	12.85±0.93	7.67±0.41*	7.38±0.86*	11.52±1.36

* indicates significant change in blood glucose level compared with normal control group (p<0.05). The results are expressed as mean ± SEM for 5 experiments

Effect of Et Ac fraction of *E. phaseoloides* on TC and TG level in Diabetic Mice: The mean serum total cholesterol and triglyceride levels of control and treated animals after 24 hours are shown in **table 3**. In case of the effects of standard drug metformin HCl and Et Ac fraction of *E. phaseoloides* on total cholesterol and triglyceride level in diabetic mice, the metformin reduced total cholesterol and triglyceride levels to 54.28% and 59.77% respectively whereas Et-Ac fraction reduced total cholesterol and triglyceride levels to 31.72%, 43.15%, 53.00% and 40.37%, 55.04%, 57.25% at the dose of 200, 250 and 350 mg/kg b.w. respectively comparing with diabetic control mice.

TABLE 3: EFFECT OF METFORMIN HYDROCHLORIDE AND ET AC FRACTION OF *ENTADA PHASEOLOIDES* ON TOTAL CHOLESTEROL AND TRIGLYCERIDE LEVEL IN NORMAL CONTROL AND ALLOXAN-INDUCED DIABETIC MICE

Treatment	24 hr	
	Total cholesterol	Triglyceride
Normal Control	5.70±0.25	2.28±0.53
Diabetic Control	13.30±0.78	6.34±0.46
D + Metformin	6.08±0.51*	2.55±0.33*
D + EtAc-EPE (200 mg/kg)	9.08±0.54*	3.78±0.16*
D + EtAc-EPE (250 mg/kg)	7.56±0.35*	2.85±0.30*
D + EtAc-EPE (350 mg/kg)	6.25±0.44*	2.71±0.44*

Values are expressed as mean ± SEM of 5 experiments. * indicates significant change compared with normal control group (p<0.05).

Effect of Et-Ac fraction of *E. phaseoloides* on Liver Enzymes (SGOT, SGPT) in Diabetic Mice: In diabetic mice SGOT and SGPT levels were raised to 80.00% and 25.85% respectively in comparison to normal mice. Following intraperitoneally administration of Et Ac fraction, SGOT and SGPT levels were significantly reduced as shown in the **table 4**.

TABLE 4: EFFECT OF DIFFERENT FRACTIONS OF *E. PHASEOLOIDES* ETHANOLIC EXTRACT ON SGPT AND SGOT LEVEL

Treatment	24 hr	
	SGOT (Unit/ml)	SGPT (Unit/ml)
Normal Control	20.0	20.5
Diabetic Control	36.0	25.8
D + Metformin	24.2*	12.6*
D + EtAc-EPE (200 mg/kg)	33.5*	18.6*
D + Pet-EPE (250 mg/kg)	30.1*	17.3*
D + Pet-EPE (350 mg/kg)	28.2*	15.5*

* indicates significant difference (p<0.05) from the diabetic control.

DISCUSSION: There are many pharmaceutical products which are available in modern medical treatment have a long history of use as herbal remedies including aspirin, opium, digitalis and quinine [16]. A large number of world's population who live in developing countries can not take the benefits of modern pharmaceuticals as those are very expensive.

Hence, phytotherapy is still a popular means of primary healthcare for which people bear a little or no cost. In addition to the use in the developing world, phytotherapy is used in the industrialized nations by alternative medicine practitioners such as naturopaths. Among the 120 active compounds currently isolated from the higher plants and widely used in modern medicine today, 80 percent show a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived [17].

Approximately 25 percent of modern drugs used in the United States have been derived from plant origins [16]. So, research on phytotherapy has got great momentum in recent years to find out noble pharmaceuticals.

The significant anti diabetic activity of Et- Ac, Pet-ether and chloroform fractions from methanolic extract of *E. phaseoloides* as shown in table 1 and 2, may be due to the presence of hypoglycemic saponins, flavonoids, and glycosides. It could be conceived that the plant extracts may also contain some biomolecules that may sensitize the insulin receptor to insulin or stimulates the β-cells of islets of langerhans to release insulin which may finally lead to improvement of carbohydrate metabolizing enzymes towards the re-establishment of normal blood glucose level.

Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic mice. Intraperitoneal administration of partitionates of methanol extract of seeds of *E. phaseoloides* resulted in a significant reduction of serum lipid levels in mice viz. triglyceride and total cholesterol. Flavonoids are known for their diverse biological activities including hypolipidemic activity resulting from their antioxidant activity. *E. phaseoloides* partitionates showed the presence of flavonoids and related phenolic compounds (**Table 5**).

TABLE 5: THE PHYTOCHEMICAL CONSTITUENTS OF THE EXPERIMENTAL PLANT FRACTIONS WERE OBTAINED BY PHYTOCHEMICAL SCREENING TESTS

Compounds→ Sample↓	Saponins	Tannins	Triterpenes	Alkaloids	Flavonoids	Glycoside
Et Ac-EPE	(+)ve	(-)ve	(-)ve	(-)ve	(+)ve	(+)ve

(-) –not detected; (+)-detected

There was a significant rise in serum GOT and GPT levels in diabetic mice, which could relate to excessive accumulation of amino acids (glutamate and alanine) in the serum of diabetic animals as a result of amino acids mobilization from protein stores [18].

The higher levels of GOT and GPT, may give rise to a high concentration of glucose. In other words, the gluconeogenic action of GOT and GPT plays the role of providing new supplies of glucose from other sources such as amino acids. Following intraperitoneally administration of active plant fraction, SGOT and SGPT levels were significantly reduced (Table 4).

These results suggest that ethyl acetate fraction of *E. phaseoloides* seeds possess an antidiabetic principle (saponins, flavonoids and glycosides) and can be useful for treatment of diabetes.

Further studies are warranted to isolate the active principle and to find out its exact mechanism of action.

CONCLUSION: This study is unique in that plant fractions cause rapid induction of hypoglycemia and hypolipidemia as well as possesses hepatoprotective effect in diabetic mice. In the light of our pharmacological studies *E. phaseoloides* seed extracts can be useful, at least as an adjunct, in the therapy of diabetes, a condition in which hyperglycemia and hyperlipidemia coexist quite often. We need further study to determine the mechanism of action and to isolate the active principles responsible for antidiabetic activity.

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