Potential Antidiabetic and Hypolipidemic Effects of Methanol Extract of *Actephila excelsa* (Dalz) Muller in Streptozocine Induced Diabetic Rats

Israt Jahan Bulbu[1*], Md. Mahbubur Rahman Tanim[1], Nowshin Akter[1]

Abstract

In Bangladesh 9% people were affected by diabetes mellitus and depend on synthetic drugs for the management of diabetes mellitus. But due to the high cost of those, poor people of rural areas of Bangladesh can't take synthetic medicine. So they were totally relying on herbal medicine to mitigate diabetes.In searching for a new herbal source to treat diabetes the study was aimed to determine anti-diabetic and hypolipidimic properties of methanol extract of Actephilaexcelsa (Dalz) Muller (Family-Euphorbiaceac) in streptozocine induced diabetic rats. In this study diabetes was induced in rats by intra-peritoneal administration of 150 mg/kg of streptozocine. Animals were treated for one week with the methanolic leaf extract of A. excelsa at doses of 250mg/kg/BW and 500mg/kg/BW and 150 mg/kg/BW metformin as standard for comparison. The antidiabetic effect was examined by measuring blood glucose level (BGL) at 0, 3, 5 and 7 days after streptozocine (STZ) treatment. Blood samples were collected on the 8th day and analyzed for triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) at sacrifice. In this study, treatment with the standard metformin at 150 mg/kg/BW reduced blood glucose level significantly ($P < 0.05$) by 65.64% compared to the untreated diabetic control group while the treatment with methanol extract of A. excelsa leaves at the doses of 250 mg/kg/BW and 500 mg/kg/BW in STZ-induced diabetes rats significantly ($P < 0.05$) reduced the blood glucose level in the treated rats by 22.09% and 25.77% compared to the untreated diabetic control group. After seven days treatment with A. excelsa at two different doses of 250 mg/kg/BW and 500 mg/kg/BW and metformin at 150mg/kg/BW in streptozotocin induced diabetic rats showed thatlow density lipid (LDL) were increased significantly ($P < 0.05$) while high density HDL decreased significantly ($P < 0.05$) compared to control rats. But for cholesterol level 500mg/kg/BW and metformin (100mg/kg) decreased cholesterol level significantly ($P < 0.05$). This study concluded that A. excelsa possesses antidiabetic property and beneficial effects on diabetic hyperlipidemia.

Key words: *Actephila excelsa (Dalz), Anti-diabetic, Hypolipidimic properties.*

Introduction

Diabetes is a condition that impairs the body's ability to process blood glucose, also known as metabolic disorder which is developed from different pathogenic mechanisms, but all results elevated blood glucose levels i.e. hyperglycemia (Burnton et al., 2011). Among four types of diabetes mellitus, type 2 diabetes mellitus is frequent which is characterized by a combination of tissue resistance of insulin along with lack of secretion from pancreas. But resistance of insulin is more regular than lack of production. This disease leads to various serious abnormalities like, cardiovascular disease, blindness, kidney failure, and lower limb amputationas well as alteration of fat metabolism accom-

1. Department of Pharmacy, Southeast University, Dhaka
*Corresponding author: Israt Jahan Bulbul, Assistant Professor, Department of Pharmacy, Southeast University, Dhaka

panied with elevated free fatty acids and triglyceride levels with lowering HDL levels. Usually it can be controlled without insulin therapy, but 30% of more efficacy can be obtained if insulin therapy is given with medication (Katzung, 2015).
According to WHO 300 million people will suffer from diabetes in 2025 in which 75% of people will be from developing or poor countries. Both oral hypoglycemic agents and insulin therapy are expensive and people from developing countries like our country, Bangladesh cannot afford the cost (Fahim et al., 2012). The location of Bangladesh is suitable for growing many varieties of plants, so that our people especially in rural areas are most familiar with the use of medicinal plants in the treatment of diseases. Many plants like *Trigonellafoenum-graecum, Cocciniaindica, Syzygium-cumini, Terminalia chebula, Azadirachta indica, Ficusracemosa, Swietenia mahagoni, Momordica charantia* are used in the treatment of diabetes in our country (Ocvirk, 2013).

Actephila excelsa (Dazl.) Muell is a shrub or tree from the family Euphorbiaceae which can be found in many countries of Asia like Bangladesh, India, Thailand, Malaysia, Vietnam etc. Limestone rich sandy areas are the main habitat of this plant. The height of the plants is 10-15 meter; trunks are 30mm thick; outer bark is greenish yellow to reddish and inner bark is green to yellow; wood is white to pale yellow; leaves are subopposite or alternate at the branch end; petiole is 2 cm enlarged in both ends; petioles are 5-95 mm; shapes can be elliptic oblanceolate or lanceolate; flowers are unisexual, male flowers clustered in axillary axis with white petals and yellow disk and female are single with short yellowish petal; fruits are capsule and compressed; each cell contains 2 seeds (Heijkoop & Welzen, 2017).

The leaves are sometimes gathered from the barren and dried leaves are used for making a pleasurable tasting tea in Sylhet and Chittagong region of Bangladesh. A fresh mucilaginous leaves juice is used three times a day for about three days to treat fever by traditional people. It is also taken by them to treat indigestion. This plant is also used traditionally to treat various disorders of gastrointestinal tract, respiratory tract, urinary tract, heart-blood circulatory system as well as skin disorders (Uddin, 2019).

In previous, a preliminary phytochemical study revealed that this plant may possess various types of flavonoids, alkaloids, tannins, carbohydrates, saponins(Ani, 2014). Sesquiterpene ɤ-elemene, β-caryophyllene, methyl salicylate, (E, E)-farnesylacetone, benzyl benzoate, sabinene, anisole and menthone have been reported to be present in the leaf oil (Dai et al., 2014). Besides two new aromatic terpenoidsepiactephilol A and actephilol A have been extracted from this plant(Ovenden et al., 2001). *A. excelsa* was previously stated to have antidiarrheal, antioxidant effects (Ani, 2014).

As this is a traditionally important medicinal plant and used in various regions of Bangladesh but has very few experimental reports of it, there is huge scope to do research of this plant. Furthermore, there is no report of its anti-diabetic and hypolipidemic effects, so the aim of this present study is to evaluate the in-vivo anti-diabetic and hypolipidemic effects of this plant.

Methods

Plant Collection

Fresh leaves of *A. excelsa which* were collected from the hill tract of Syllhet division of Bangladesh were then identified from National Herbarium, Dhaka, Bangladesh and a herbarium document was kept on there (Accession number: 43912). The name *A. excelsa* was also checked in https://mpns.science.kew.org/ and confirmed the acceptance of this name.

Extraction

The fresh leaves of *A. excelsa* were washed under running water and dried on sunlight for a week then grinded into coarse powder. About 250 grams of powder was taken in a glass bottle and filled with 1.5 litre of methanol and then was soaked for a week with a continuous one hour shaking for each day. The liquid then was filtered twice with cotton and the filtrate was then placed on a rotary evaporator and the amount was reduced to 100ml at 45°C. The extract was taken in a beaker and placed on a water bath at 45°C until it became gummy and sticky.

Animals

Long Evan rats of 60 grams were purchased from icddr,b Dhaka, Bangladesh and fed them to gain minimum weight to run the test. These animals were kept in a standard rat case at 25°C and a proper day- night circle was maintained.

Acute Toxicity Test

Eight male and female rats of 70-80 grams were divided into two groups and they were fasted for twelve hours. At the thirteenth hour these two groups received 250 mg/kg and 500 mg/kg methanolic extracts of *A. excelsa* (MEAE) respectively. For the first three hour of treatment the animals were observed for any toxic syndrome like increased motor activity, coma or death. The death rate was also observed for the next four days (Kumar, et al., 2013).

Antidiabetic Assay

Twenty rats of both sexes were divided into five groups as

- Group-I was normal control
- Group-II was untreated diabetes
- Group-III was standard control received metformin (150mg/kg)
- Group-IV was received MEAE 250 mg/kg
- Group-V was received MEAE 500 mg/kg

Animals of group II to V were fasted for 12 hours before treatment. Then the amount of streptozotocin was calculated (55mg/kg/BW) for each animal and dissolved on 0.5ml sterile normal saline solution and given through intra-peritoneal route. The animals were also given 10% glucose solution to avoid hypoglycemia. After 73 hours of streptozotocin induction the blood glucose level was checked from the tail vein by using a digital glucometer and animals which had above 7.0 mmol/L blood glucose level were selected for further study. The amount of metformin hydrochloride and crude MEAE were calculated according to the body weights of the animals and 0.5 ml solution was prepared for each animal in sterile saline. The animals received their treatment orally for seven consecutive days and after three, five and seven days of first dose administration the blood glucose levels were examined. Percent of inhibition of diabetes was calculated by the following equation:

% Inhibition of Blood Glucose Level (BGL) = (BGLBT − BGLAT)/BGLBT × 100,
BGLBT = Blood glucose level before treatment
BGLAT = Blood glucose level after treatment

On the seventh day the animals were anesthetized by chloroform and 3 ml blood were taken by direct cardiac puncture from each animal. The blood was centrifuged at 4000 rpm for 20 minutes and the serum was preserved for lipid profile analysis (Saleh et al., 2013).

Lipid Profile Analysis

Serum lipid markers- triglyceride, cholesterol, HDL & LDL were measured by a biochemical analyzer by manufacturer provided kit and according to their methods (Kumar et al., 2013).

Data Analysis

The statistical analysis was carried out by Graph Pad Prism 8 and all values are expressed as **mean** ± SEM (n=4). A one-way analysis of variance (ANOVA) followed by a Dunnet test was performed to compare multiple groups. Values were considered statistically significant at $P<0.05$, $P<0.01$, $P<0.001$.

Results

Result of Acute Toxicity Test

On fourth dayof acute toxicity test, none of the eight rats were observed as died after taking the extracts of *A. excelsaat* the doses of 250 mg/kg and 500 mg/kg b.w. and no sign and symptoms of toxicity were observed during that four days period of treatment.

Results of Antidiabetic Test

All the test groups including control, untreated diabetic control, standard and extracts at two different doses were subjected to determine blood glucose level at 3rd, 5th and 7th days. In this study, treatment with the standard metformin at 150 mg/kg/BW reduced blood glucose level significantly ($P < 0.05$) by 65.64% compared to the untreated diabetic control group. The treatment with methanol extract of *A. excelsa* leaves in STZ-induced diabetes rats at 250 mg/kg/BW significantly ($P < 0.05$) reduced the blood glucose level in the treated rats (Fig. 1) by 22.09% while at a dose of 500 mg/kg/BW, blood glucose level was decreased significantly ($P < 0.05$) by 25.77% compared to the untreated diabetic control group.

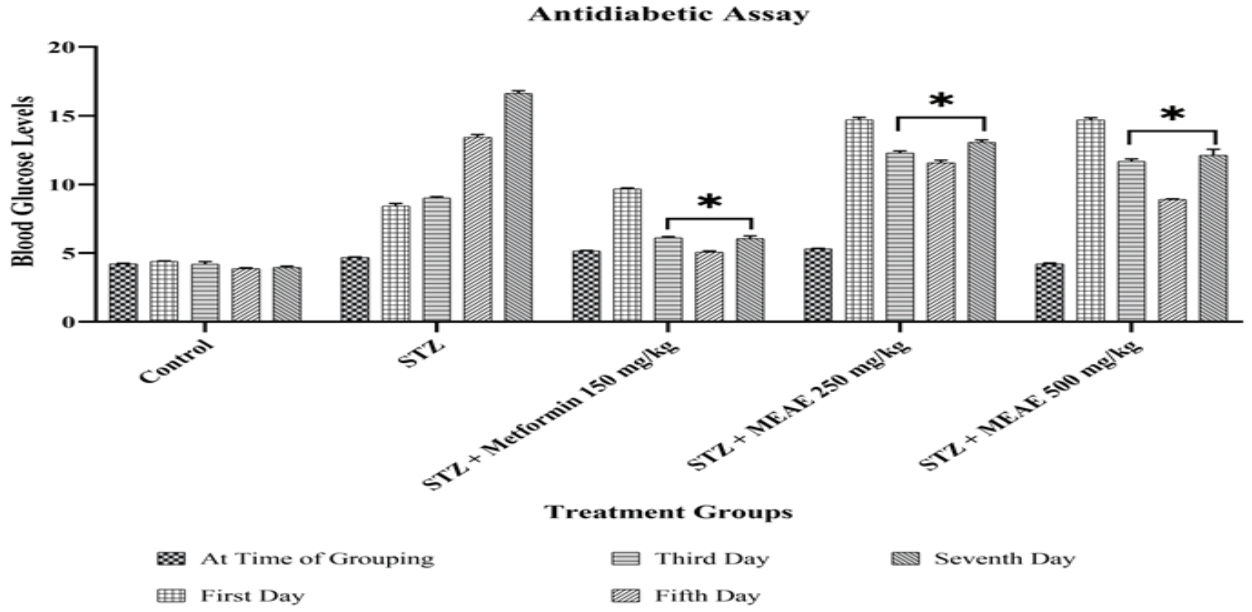
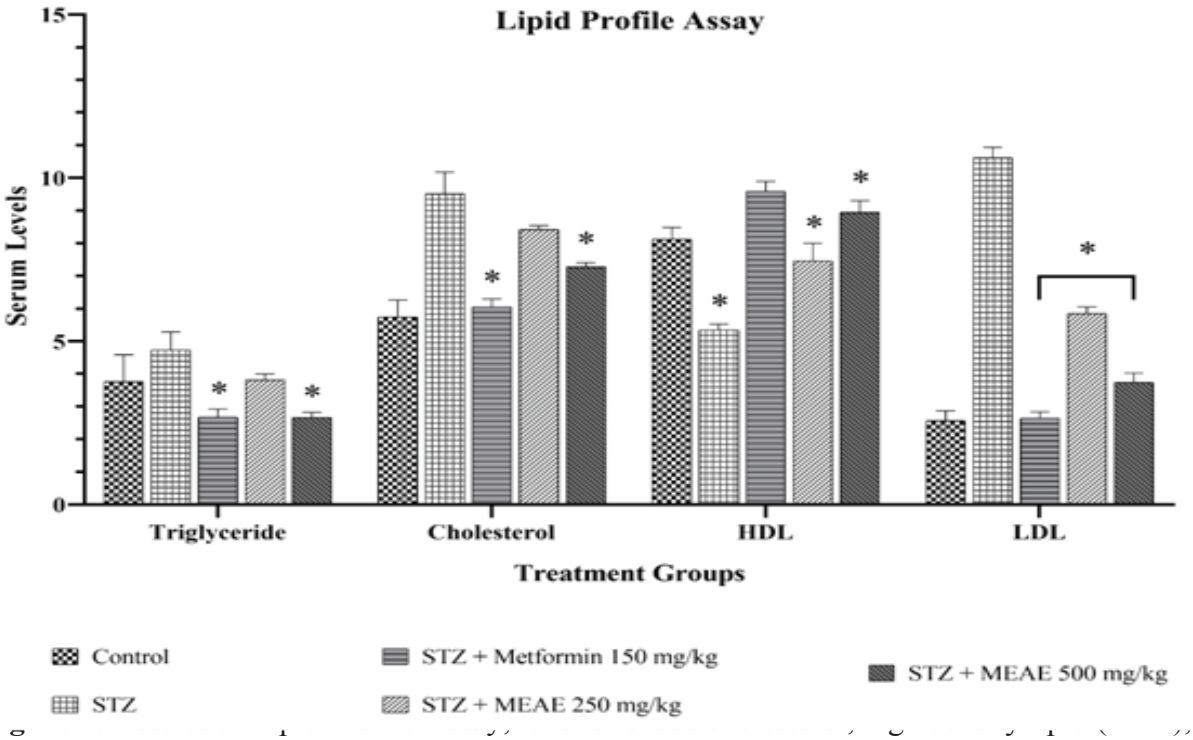


Figure 1: Results of blood glucose level (BGL) after 7 days treatment with *A. excelsa* at 250 mg/kg/BW and 500 mg/kg/BW in STZ induced diabetic rats, Here, * values are statistically significant at $P<0.05$

Results of Lipid Profile Test

The level of cholesterol was increased after diabetes induction by streptozotocin as compared to control. After 7 days treatment with standard metformin at a dose of 150 mg/kg/BW, cholesterol level was significantly ($P < 0.05$) decreased. Between two doses of A. excelsa 500 mg/kg/BW showed a reduced cholesterol level in a significant manner at $P < 0.05$.
Regarding HDL, its level was reduced after administration of streptozotocin in rats. But after the entire treatment, HDL level was found increased (significantly at $P < 0.05$) for the both doses of the plant extract.

The LDL level was drastically increased with the increased blood glucose level due to the administration of streptozoocin. But the LDL level was reduced significantly ($P < 0.05$) for standard as well as two different doses of *A. excelsa* (250 mg/kg/BW and 500 mg/kg/BW).



density lipid (LDL) after 7 days treatment with *A. excelsa* at 250 mg/kg/BW and 500 mg/kg/BW in STZ induced diabetic rats, Here, * values are statistically significant at $P<0.05$

Discussions

Methanol extract of *A. excelsa* was evaluated for its *in-vivo* hypoglycemic and hypolipidemic activities in STZ induced Long Evan rats as compared to the standard metformin, a hypoglycaemic and hypolipidemic drug to verify its effect. Streptozotocin is a naturally occurring alkylating agent, chemically known as glucosamine–nitrosourea compound, used for the treatment of metastatic cancer of the pancreatic islet cells. In this study STZ was used at a high single dose to induce type 2 diabetes. STZ not only damages DNA but may also induce activation of Poly (ADP-ribose) polymerase, PARP which plays an important role for diabetes induction (Lenzen, 2008; Szkudelski, 2001). Additionally, a toxic amount of nitric oxide is liberated by STZ that prevents aconitase activity and results in DNA damage (Wang & Gleichmann, 1998; Schnedl et al., 1994)
In this study, the methanol extract of *A. excelsa* leaves in STZ-induced diabetic rats at 250 mg/kg/BW and 500 mg/kg/BW significantly ($P < 0.05$) reduced the blood glucose level compared to the

untreated diabetic control group after 7 days treatment. The % inhibitions of blood glucose level were 22.09% and 25.77% for both the increasing doses, while for the standard metformin it was 65.64%. The hypoglycemic activity may be due to the terpene richness of this plant (Dai et al., 2014; Ovenden et al., 2001). Previously a number of terpenoids have been reported as hypoglycemic and they produce their hypoglycemic effect by developing insulin resistance, regularizing the plasma glucose and insulin levels and glucose metabolism (Putta et al., 2016).

Many type 2 diabetic patients generally have mild hypertriglyceridemia because of the excess concentration of TG-rich lipoproteins in the liver, accompanied with reduced high-density lipoprotein (HDL) cholesterol levels. Hyperlipidemia leads to cardiovascular morbidity and mortality. Uncontrolled hyperlipidemia follows micro vascular complications (Samarghandian et al., 2013). Some oral hypoglycemics like sulfonylureas or insulin can treat this hypertriglyceridemia as well as decrease HDL cholesterol (Abbate & Brunzell, 1990). In this test, the levels of triglyceride (TG), total cholesterol (TC), LDL levels were increased and HDL levels were decreased after streptozotocin injection compared to control and untreated diabetic groups.The studied plant extract exhibited very good hypolipidemic activity after 7 days treatment and thus plays a vital role in caring for the heart against cardiovascular disease. Two doses of *A. excelsa* 500 mg/kg/BW showed a reduced cholesterol level in a significant manner at $P < 0.05$ while HDL level was found increased (significantly at $P < 0.05$) for the both doses of the plant extract. But the LDL level was reduced significantly ($P < 0.05$) for standard as well as two different doses of *A. excelsa* (250 mg/kg/BW and 500 mg/kg/BW).

Conclusion

The present conclusions include significant hypoglycemic and hypolipidemic activities of *A. excelsa* in diabetic micethat were treated by STZ to persuade diabetes. This finding provides evidenced based support for the use of *A. excelsa* to treat diabetic patients associated with diabetes induced hyperlipidemia but require more intensive study regarding mechanism for its hypoglycemic effect as well as to identify the phytoconstituents responsible for this effect.

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