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Antihyperglycemic and antihyperlipidemic activity of ethanol extract of garlic *(Allium sativum)* in streptozotocin-induced diabetic mice

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Abstract

The present study was conducted to evaluate the hypoglycemic and hypolipidemic effects of ethanol extract of *Allium sativum* (EEAS) locally grown in Bangladesh. Male Swiss albino mice were divided into four groups as normal control, diabetic control, EEAS-treated and glibenclamide-treated diabetic mice. Diabetes was induced in mice by intraperitoneal administration of streptozotocin (50mg/kg of body weight) and treatment was started 14 days after diabetes induction. In extract-treated mice, EEAS was orally given at a dose of 200mg/kg for 28 days and its effect on blood glucose level and body weight was determined on a weekly basis. On completion of the treatment, fasting blood sample was collected to estimate a range of indicators including blood lipid profile, serum glutamate-oxaloacetate transaminase (SGOT) and serum glutamate-pyruvate transaminase (SGPT). Oral administration of EEAS in diabetic mice resulted in significant ($P<0.01$ to $P<0.001$) reduction in blood glucose level which was accompanied by an increase in body weight. EEAS treatment significantly ($P<0.05$ to $P<0.01$) reversed abnormal status of lipid profile towards near normal levels compared to diabetic control mice. In addition, there was a significant ($P<0.001$) decrease in the activities of SGOT and SGPT in extract-treated diabetic mice compared to untreated diabetic mice indicating the protective role of the extract against liver damage. The findings of this study suggest that ethanol extract of garlic grown in Bangladesh possesses the capability of managing hyperglycemia and complications of diabetes in streptozotocin-induced diabetic mice. Therefore this plant may be considered as a potential resource for the development of alternative medicine in the management of diabetes mellitus.

Keywords: allium sativum, antihyperglycemic, antihyperlipidemic, streptozotocin

1. Introduction

Morbidity and mortality due to diabetes mellitus (DM), a chronic metabolic disorder characterized by hyperglycemia, is increasing worldwide and is considered as the third cause of death after cancer [1, 2]. According to the report of International Diabetes Federation (IDF), the worldwide prevalence of diabetes mellitus at the end of 2013 was 382 million, and it is projected to reach 592 million by 2035 [3]. It has been reported that diabetes tends to increase low-density lipoprotein cholesterol and decrease high-density lipoprotein cholesterol levels in blood that leads to coronary occlusions and blocks. Currently used synthetic hypoglycemic drugs have been suspected to possess adverse effects like hypoglycemia, gastro-intestinal disturbances, renal toxicity and hepatotoxicity [4]. Therefore, the search for new drug with better efficacy and minimum or no adverse effects is an active field. Experimentally, various medicinal plants have been found to have antidiabetic activity [5].

Garlic (*Allium sativum* L., family: Alliaceae) has played important dietary and medicinal roles throughout the ages. It has been evaluated for a number of purposes, including treatment of hypercholesterolemia, hypertension, diabetes, rheumatoid arthritis, cold or the prevention of atherosclerosis and the development of tumors. Many available publications have indicated the possible antioxidant, antibacterial, anti-hypertensive and anti-thrombotic properties of various garlic varieties [6, 9]. In Bangladesh, a local variety of garlic is cultivated abundantly, but there is no available scientific report regarding the hypoglycemic and hypolipidemic properties of this variety. Hence, the present study investigated the hypoglycemic and hypolipidemic effects of locally grown garlic extract in streptozotocin-induced diabetic mice.

2. Materials and Methods

2.1 Collection of plant material and authentication

For the present study, local variety garlic of Bangladesh was collected from the area of Rajshahi (north-western part of Bangladesh) and authenticated by the Department of Botany, University of Rajshahi, Bangladesh.

2.2 Preparation of extract

About 100gm of the powdered garlic material was taken in a clean, round bottomed glass bottle and soaked in 500 ml of ethanol. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 15 days accompanying occasional shaking and stirring. The resulting extracts were filtered through Whatman No. 1 filter paper. Afterwards, the solvents were evaporated under reduced pressure at 40˚C using rotary evaporator. Finally, the residues were kept in small sterile bottles under refrigerated conditions until used.

2.3 Animal care

Male Swiss albino mice (weighing about 25-30gm) collected from the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR'B), Dhaka were housed in polypropylene cages in well-ventilated rooms under standard conditions (temperature 25±2ºC; humidity 55±5% with 12h light/dark cycle) and were allowed free access to standard dry pellet diet and water. The approval and permission of using mice model in this study were obtained from the Institute of Biological Sciences, University of Rajshahi, Bangladesh.

2.4 Induction of diabetes

Diabetes was induced in overnight fasted mice by a single intra-peritoneal injection of streptozotocin (50mg/kg of body weight) in a 0.1M sodium citrate buffer (pH-4.5). The development of hyperglycemia in mice was confirmed by fasting (16 hour) blood glucose measurement in the tail vein blood 48 hours after streptozotocin administration with a portable glucometer. After 14 days of diabetes induction, blood glucose level was again determined and mice with a blood glucose level greater than 16.0mmol/L were included in this study.

2.5 Experimental design

The animals were randomly divided into four groups and each group consisted of six mice:
Group 1: Untreated normal control mice
Group 2: Untreated diabetic control mice
Group 3: Diabetic mice treated with ethanol extract at a dose of 200mg/kg body weight/day for 28 days
Group 4: Diabetic mice treated with glibenclamide (5mg/kg) for 28 days
Collection of blood samples from the tail veins as well as measurement of body weight of each mouse was carried out before the start of the treatment and on week 1, 2, 3 and 4 during the treatment. At the end of experiment period (after 28 days), mice were sacrificed after overnight fasting by anesthetizing with diethyl ether and blood was collected from the heart and immediately stored at -20°C till further analysis.

2.6 Estimation of biochemical parameters

Estimation of blood glucose was performed by glucose oxidase-peroxidase method [10]. Serum lipid profile such as triglyceride (TG), total cholesterol (TC), and high density lipoprotein (HDL) was measured using commercially available kits. Serum low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were determined according to the Friedewald formula: VLDL = TG/5; LDL = TC − (HDL + VLDL). Hepatic enzymes such as SGPT and SGOT were also measured using commercially available kits. Hitachi 7180 automatic analyzer (Hitachi, Tokyo, Japan) based on the standard enzymatic procedures was used to estimate all these biochemical parameters. Atherogenic index (AI) was calculated using an equation developed by Friedewald [11]. Atherogenic index (AI) = (serum total cholesterol – HDL)/HDL.

2.7 Statistical analysis

All values were expressed as mean ± SD (Standard Deviation). Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by Dunnett's t test using SPSS software of 16 version. $P<0.05$ were considered to be statistically significant.

3. Results

3.1 Effect of EEAS on blood glucose and body weight

Administration of streptozotocin in mice significantly ($P<0.001$) increased the blood glucose levels compared to the normal group. The oral treatment of EEAS at a dose of 200mg/kg resulted in a significant ($P<0.01$ to $P<0.001$) reduction in the blood glucose level of streptozotocin-induced diabetic mice compared to diabetic control mice (Table 1). At the end of 4 weeks, EEAS administration reduced the blood glucose level by approximately 54% compared to diabetic control mice. Body weight of mice was reduced significantly ($P<0.001$) after administration of streptozotocin compared to the normal mice. In diabetic mice, treatment of EEAS at the same dose significantly ($P<0.05$ to $P<0.01$) increased body weight compared to diabetic control mice. These results were quite comparable to the data obtained from glibenclamide-treated mice (Table 2).

Table 1: Hypoglycemic activity of EEAS in streptozotocin-induced mice

Group	Blood glucose (mmol/L)				
	Initial day	1st week	2nd week	3rd week	4th week
Normal control	4.27±0.86	5.1±0.7	4.83±0.67	4.3±0.72	4.37±0.67
Diabetic control	19.16±0.95^a	20.33±1.8^a	19.83±2.2^a	21.6±1.23^a	20.4±1.65^a
EEAS-treated	19.7±0.85^a	17.67±0.49	14.46±0.67^b	10.46±1.14^b	9.33±0.80^c
Glibenclamide	20.3±1.35^a	12.33±2.05^b	7.43±0.49^c	6.47±0.91^c	5.67±0.67^c

Results are expressed as mean±standard deviation (n=6). $^aP<0.001$compared with normal control; $^bP<0.01$ and $^cP<0.001$ compared with diabetic control

Table 2: Effect of EEAS on body weight change in streptozotocin-induced mice

Group	Body weight (gm)				
	Initial day	1st week	2nd week	3rd week	4th week
Normal control	28.3±1.05	29.2±0.62	29.83±0.55	30.13±0.35	32.33±1.8
Diabetic control	27.13±0.65	26.1±0.1	25.97±0.15^a	25.3±0.26^a	23.8±0.64^a
EEAS-treated	27.27±0.47	26.2±0.27	27.2±0.89	27.37±0.45^c	28.3±0.62^c
Glibenclamide	27.53±1.35	26.9±0.44	27.7±0.61^b	28.53±0.45^d	29.13±1.05^d

The data are given as mean±standard deviation (n=6). $^aP<0.001$ compared with normal control; $^bP<0.05$, $^cP<0.01$ and $^dP<0.001$ compared with diabetic control.

3.2 Effect of EEAS on lipid profile

In streptozotocin-induced diabetic mice, TG, TC, LDL and VLDL levels were increased whereas HDL level was decreased significantly (P<0.05 to P<0.001) compared to that of normal mice. In diabetic mice, administration of EEAS at a dose of 200mg/kg showed significant (P<0.05) reduction in elevated TG, TC, LDL and VLDL levels compared to diabetic control mice. EEAS-treated group also exhibited a significantly (P<0.05) increased level of HDL compared to diabetic control mice (Table 3).

Table 3: Hypolipidemic effect of EEAS in streptozotocin-induced mice

Group	Serum lipid profile (mg/dL)					AI
	TC	TG	HDL	LDL	VLDL	
Normal control	90.83±3.88	85.67±6.75	44.9±5.15	28.8±7.4	17.13±1.35	1.04±0.25
Diabetic control	133.7±9.03[a]	127.33±26.75[a]	35.27±4.28[b]	72.96±2.46[c]	25.47±5.35	3.04±0.27[a]
EEAS-treated	110.9±12.28	78±8.67[d]	45±1.04[d]	50.3±10.10[d]	15.6±1.73[d]	1.46±0.22[e]
Glibenclamide	110.17±8.21[d]	103.67±11.42	45.53±3.37[d]	43.9±5.46[e]	20.73±2.29	1.42±0.17[e]

Results are expressed as mean±standard deviation (n=6). [a]P<0.001, [b]P<0.05 and [c]P<0.01 compared with normal control; [d]P<0.05 and [e]P<0.01 compared with diabetic control; AI: Atherogenic index.

3.3 Effect of EEAS on SGPT and SGOT level

Table 4 represented the effect of EEAS on serum SGOT and SGPT activities in diabetic mice. The above two parameters were significantly (P<0.001) altered in streptozotocin-induced diabetic mice compared to that of normal mice. In diabetic mice, significant (P<0.001) reduction in SGOT (around 45% reduction) and SGPT (around 58% reduction) level was achieved by the administration of EEAS at a dose of 200mg/kg for 28 days compared to diabetic control mice and these results were almost similar to that of glibenclamide-treated mice (5mg/day).

Table 4: Effect of EEAS on SGOT and SGPT level in streptozotocin-induced mice

Group	SGOT (U/L)	SGPT (U/L)
Normal control	130.63±2.44	61.53±1.10
Diabetic control	245.43±2.63[a]	141.47±0.91[a]
EEAS-treated	133.33±3.8[b]	58.87±0.78[b]
Glibenclamide	135.5±2.51[b]	64±0.32[b]

The data are expressed as mean±standard deviation (n=6). [a]P<0.001 compared with normal control; [b]P<0.001 compared with diabetic control.

4. Discussion

Streptozotocin was used in the current investigation to induce diabetes mellitus in experimental mice. At low dose, streptozotocin (50mg/kg) partially destroys the beta cells of pancreas resulting in insufficient insulin production causing type2 diabetes [12]. It has been widely used to induce diabetes mellitus for its selective beta-cell cytotoxicity, and it is less toxic than alloxan and allows a consistent maintenance of diabetes mellitus [13, 14]. Induction of diabetes in mice resulted in decreased blood glucose level and lowered body weight compared to the normal mice. The reduced body weights observed in diabetic mice induced by streptozotocin administration may be due to muscle wasting and loss of tissue proteins [15, 16]. The orally administered EEAS remarkably compensated the altered blood glucose level and body weight in the diabetic mice. This may results from the promotion in insulin production from the remnant pancreatic beta cells, consequently resulting in improvement in glycemic control.

It has been demonstrated that accumulation of lipids such as total cholesterol and triglycerides occurs in diabetic patients due to insulin insufficiency [17]. The increased mobilization of free fatty acids from the peripheral fat depots is mainly responsible for the abnormal high concentration of serum lipids in the diabetic patients [18]. In the present study, EEAS treatment of the diabetic mice satisfactorily reduced the TC, TG, LDL, and VLDL levels with an increase of HDL compared to untreated diabetic mice. This may be due to the insulinotropic activity or insulin secretagogue property of the extract. The observed increase in atherogenic index of EEAS-treated diabetic mice compared to diabetic control mice is due to increase in HDL levels. HDL is well known for its important role in the transport of cholesterol from peripheral cells to the liver and is considered to be a vital cardio protective lipid [19]. The present study results were in agreement with findings of Thomson *et al*., (2016) which also demonstrated hypoglycemic and hypolipidemic activities of aged garlic extract in diabetic rat model [20].

In diabetic condition, impairment of carbohydrate, lipid and protein metabolisms together with oxidative stress are likely to affect hepatic function. Hence our current study also investigated the protective activity of EEAS against hepatic damage caused by diabetes mellitus. In diabetic mice, an increase in SGPT and SGOT enzyme activities reflects active liver damage and has been reported to be associated with increased gluconeogenesis and ketogenesis [21]. Treatment of diabetic mice with EEAS resulted in considerable reduction in the activities of these two enzymes in serum compared to diabetic control mice.

5. Conclusion

The above data confirm that local variety garlic of Bangladesh possesses potent hypoglycemic, hypolipidemic and hepatoprotective properties in diabetic mice; therefore, it can be used to prevent diabetes mellitus and the associated complications. However, the nature of the active principle(s) responsible for all these beneficial effects requires further investigation.

6. Conflict of Interest Statement

We declare that we have no conflict of interest.

7. Acknowledgements

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