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Research Article

Asparagus racemosus Linn. Potentiates the Hypolipidemic and Hepatoprotective Activity of Fenofibrate in Alloxan-Induced Diabetic Rats

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Abstract: Diabetes mellitus is strongly connected with changes in lipid profile and also can cause damage of several organs like liver over a long period of time. The purpose of this study was designed to evaluate the hypolipidemic and hepatoprotective effects of ethanolic root extracts of *Asparagus racemosus* (EEAR) Linn. alone and in combination with a lipid lowering agent (fenofibrate) in alloxan-induced diabetic rats. Diabetes was induced in male Wister albino rats by the administration of single intra-peritoneal injection of alloxan monohydrate (120 mg/kg b.w.). Two different doses of EEAR (200 and 400 mg/kg b.w.) alone, fenofibrate (30 mg/kg b.w.) and a combination of EEAR (200 mg/kg b.w.) with fenofibrate (30 mg/kg b.w.) were administered orally for the period of 14 days. After the treatment period, hypolipidemic and hepatoprotective effects were determined by examining serum biochemical markers including total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL), serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT) and total protein (TP) with the aid of commercially available kits. The survival rate, body weight and organ weight were also measured. The ingestion of EEAR considerably ($p < 0.05$, $p < 0.01$, $p < 0.001$; $p < 0.05$, $p < 0.01$) modified the activity of TC, TG, LDL, VLDL and HDL cholesterol levels when compared to the disease control and fenofibrate treated rats. The administration of combination therapy significantly ($p < 0.001$; $p < 0.001$) improved the activity of TC, TG, LDL, VLDL and HDL levels when compared to that of disease control and fenofibrate treated rats. The rats treated with EEAR markedly ($p < 0.05$, $p < 0.01$, $p < 0.001$; $p < 0.05$) reduced the level of SGOT, SGPT and TP as compared to the disease control and fenofibrate treated rats. The suggested combination therapy significantly ($p < 0.001$; $p < 0.001$) decreased the level of SGOT, SGPT and TP when compared to that of disease control and fenofibrate treated rats indicated amelioration in liver dysfunctions. The maximum survival rate was 100% found in combination therapy. During treatment period, it was observed that the considerable ($p < 0.01$, $p < 0.001$; $p < 0.05$, $p < 0.01$, $p < 0.001$) changes in the body weight were found in the EEAR treated rats and combination therapy on 10th and 14th day as compared to that of disease control and fenofibrate treated rats. In case of organs weight, the weight of the liver and weight of the pancreas were significantly ($p < 0.001$; $p < 0.05$, $p < 0.01$, $p < 0.001$) decreased in the rats treated with highest dose of EEAR (Alx+ EEAR 400) and combination therapy when compared to the disease control and fenofibrate treated rats. The current study demonstrates that combination therapy of EEAR and fenofibrate was more effective than that of monotherapy in controlling diabetes mellitus associated with cardiovascular diseases and hepatic dysfunction in alloxan-induced diabetic rats.

Keywords: Diabetes Mellitus, *Asparagus racemosus*, Hypolipidemic Activity, Hepatoprotective Activity, Fenofibrate, Combination Therapy

1. Introduction

Abnormal heightened levels of any or all lipids and or lipoproteins in the blood is defined as hyperlipidemia which is engendered by either genetic (primary hyperlipidemia) or a poor diet and other particular factors (secondary hyperlipidemia) arises because of other underlying reasons such as diabetes [1]. In addition to hyperglycemia, there are other two factors, hypercholesterolemia and hypertriglyceridemia are also common complications of diabetes mellitus [2]. Hyperlipidemia's frequency in diabetes is highly depending on the type of diabetes and its degree of control. Diabetes mellitus (DM) is the most common demonstrated metabolic disorder that is occurred in human due to the high consumption of carbohydrates and lipids, which has been affecting millions of people globally [3]. DM is a metabolic disorder characterized by hyperglycemia as well as hyperlipidemia [4]. Hyperlipidemia basically an elevated level of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein (LDL) cholesterol, very low-density lipoprotein (VLDL) cholesterol along with a lower level of high density lipoprotein (HDL) cholesterol, is the forecaster of coronary artery disease, fatty liver disease and carcinogenesis, which correlates with the formation of reactive oxygen species (ROS) [5, 6]. Over 70% of patients with type 2 diabetes mellitus had one or more types of dyslipidemia [7]. Hyperlipidemia is a primal risk factor for cardiovascular diseases (CVD) besides, accountable for the initiation and progression of atherosclerotic impasse [8]. Hyperglycemia, obesity, dyslipidemia, insulin resistance, hypertension, inflammation, autonomic dysfunction and diminished vascular responsiveness are contributors of CVD risk in DM. High blood glucose level continuously generating ROS and superoxide anions, that further aggravates the diabetic complication by impairing the protein, deoxyribonucleic acid and carbohydrate, which leading to increasing the oxidative stress [9]. The concentration of the free radical production could be favorably reduced through appropriate dietary intake and drug therapy and thus less chance of diabetes and diabetic associated CVS disorder [10]. Hence, it is dominant to control not only blood glucose levels, but also blood lipid levels.

The liver is a large organ and its main function is managing and controlling carbohydrates, lipids and protein metabolism. To maintain normal blood glucose levels by taking and storing glucose in the form of glycogen (glycogenesis), cleavage of glycogen into glucose (glycogenolysis), and forming glucose from non-carbohydrate sources for example amino acids (gluconeogenesis) are some other functions of liver [11, 12]. Various studies have shown that alloxan has deleterious effects on liver and kidney [12, 13]. Disruption in liver function that is demonstrated with increasing in the alanine and aspartate aminotransferases (ALT and AST) have been reported after one week of alloxan injection [14, 15]. The enzymes, gamma-glutamil transferases (γGT) and bilirubin are measured for investigating liver function [15]. Aminotransferases are the markers of the healthy hepatocyte [16]. Liver has a major role in maintaining postprandial normal glucose concentration and it is the main site of insulin clearance [17].

Alloxan is commonly used to instigate diabetes mellitus in experimental animals as it causes severe necrosis of pancreatic β-cells with the consequent lower level of insulin secretion [18-20]. Oxidative stress, which is highly induced by alloxan is also another possible mechanism of its diabetogenic action [21, 22]. Lipids play an essential role in maintaining the probity of biomembrane structure and functions [23]. Modification in cholesterol phospholipid molar ratio results in greater red cell membrane permeability, fragility and reduced fluidity. Modified lipids and lipoprotein metabolism in chronic diabetes mellitus are associated with the pathogenesis of atherosclerosis and other cardiovascular diseases [24]. Aberration in lipoprotein and plasma lipid patterns due to insulin insufficiency has been well documented, in both type I and type II DM [25]. Moreover, the increment of the activities of AST, ALT and TP in plasma may be primarily because of the leakage of these enzymes from the liver cytosol into the blood stream which provides an indication of the hepatotoxic effect of the alloxan [26].

Medicinal plants have an important role in the discovery of new counteractive agents and received much more consciousness as the source of biologically active substances including, antioxidant, antihyperglycemic and antihyperlipidemic agents [27]. Drugs from natural source are less toxic and are considered to have fewer side effects than synthetic drugs [28]. Medicinal plants exhibit natural remedies that are considered to be effective and safe as alternative treatments for hyperglycemia with hyperlipidemia and liver toxicity [29]. The world health organization (WHO) estimates that 80% of the population in some countries use medicines that are from natural sources, for a number of specific aspects of primary health care [30, 31]. In recent years, investigation of herbal medicines has become deliberately influential in the search for new, effective and safe therapeutic agent to treat diabetes associated with hyperlipigemia and liver dysfunction.

The plant *Asparagus racemosus* (AR) Linn. is commonly known in Bengali as Satamuli, Satavari, Satawar belongs to the family Liliaceae found at low altitudes throughout Bangladesh, India, Asia, Australia and Africa [32]. It grows one to two meters tall and prefers to take root in gravelly, rocky soils high up in piedmont plains, at 1,300–1,400 meters elevation [33]. It is widely used for the treatment of diarrhea, dysentery, rheumatism, nervous breakdown, and is thought to be an aphrodisiac [34]. Some investigation reports indicate that the pharmacological activities of AR root extract include antiulcer, antioxidant, and antidiarrheal, antidiabetic and immunomodulatory activities [35]. Root of AR has different properties including emollient, cooling, nervine tonic, constipating, galactogogue, and aphrodisiac, diuretic, rejuvenating, carminative, stomachic, antiseptic and as tonic [36]. Beneficial effects of the root of AR are suggested in nervous disorders, dyspepsia, diarrhea, dysentery, tumors, inflammations, hyper dipsia, neuropathy, hepatopathy, cough, bronchitis, hyperacidity and certain infectious diseases [35, 37]. It has also been claimed that the root of this plant was used by traditional healers for various disease state [38]. AR possesses the chemical constituents such as flavonoids, oligosaccharides, amino acids, sulphur-containing acids and steroidal saponins [39]. Various reports suggest that polysaccharides derived from the plant exhibit antioxidant as well as radioprotective properties [40, 41]. The polysaccharide kreskin also has been shown to have inhibitory effects on the oxidation of low density lipoprotein LDL [42, 43].

A preliminary study was shown that ethanolic extracts of AR has antidiabetic and antihyperlipidemic activity in alloxan induced diabetic animal's model [32, 44]. The combined effect of lipid lowering drug (fenofibrate), with AR had determined for the first time, in this study. The current study was designed to investigate the effect of ethanolic extract of AR (EEAR) either potentiates the hypolipidemic and hepatoprotective activity of the fenofibrate or not in alloxan-induced diabetic rats.

2. Methodology

2.1. Chemicals and Drugs

Alloxan monohydrate was brought from Explicit Chemicals, Pvt. Ltd, Pune, India. Ez Smart 168 (Tyson Bioresearch, Inc. Chu-Nan, Taiwan) glucose test meter was used for investigating the blood glucose level. Total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL) kits were obtained from Human Gesellschaft fur Biochemical mbH-Wiesbaden, Germany. Serum glutamate oxaloacetate transaminases (SGOT), serum glutamate pyruvate transaminases (SGPT) and total protein (TP) kits were acquired from Linear chemicals-Barcelona, Spain. The drug, fenofibrate was the generous gift sample from Beximco pharmaceuticals limited, Dhaka, Bangladesh. All other chemicals were purchased from local sources and were of analytical grade.

2.2. Collection, Identification, Drying and Grinding of Plant Material

In this experiment roots of AR were collected from the neighboring area of Kurigram, Bangladesh, in February, 2014. After collection roots were thoroughly washed with water. The plant was recognized by specialist of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. Accession number: DACB-39527 for AR. The collected roots were washed and sun dried under the shadow for two weeks. By using a suitable grinder the dried roots were ground into a coarse powder.

2.3. Preparation of Plant Extract

The powdered roots (500 g) of the AR were taken in an amber colored glass bottle and soaked in 2.5 liter of 98% ethanol at room temperature. The bottle was stored at room temperature and permitted to stand for 15 days with occasional shaking and stirring. Afterward, the extracts were filtered through cotton filter and then through Whatman filter paper (No. 1). Then the liquid filtrates were concentrated and evaporated to dry at temperature 40°C by using a rotary evaporator under reduced pressure to get the crude extract 12.31 g, ultimately the dried crude extracts were kept in a refrigerator at 4°C until further experiment.

2.4. Acute Oral Toxicity Study

An acute oral toxicity study was carried out for the EEAR as per Organization for Economic Co-operation and Development- 423 guidelines (acute toxic class method) [45]. Healthy male Wister albino rats were arbitrarily divided into six groups with 5 animals in each group were used for acute oral toxicity study. The rats were kept fasting overnight with supplementation of water before oral dosing, then the EEAR was administered orally with increasing doses [100, 200, 500, 1000, 1500, and 2000 mg/kg of body weight (b.w.)] by using intragastric tube. The rats were carefully observed constantly for 24 hrs for behavioral and any other adverse change and consequently for any lethality.

2.5. Preparation of Dosage of Fenofibrate and Plant Extract

The solution of fenofibrate was prepared by dissolving with dimethyl sulfoxide (DMSO) and administered at the dose of 30 mg/kg b.w. The dosage of fenofibrate was selected based on literature review [46]. A suspension of EEAR was prepared by normal saline (pH 7.4) and administered orally to rats at 200 and 400 mg/kg b.w.

2.6. Animals

Weight of about 140 to 170g, healthy, adult male Wister albino rats were purchased from the animal house of Jahangirnagar University, Dhaka, Bangladesh. The rats used in the studies were kept in hygienic individual polyethylene cages in a well-ventilated room and they were maintained under standard condition (12 hrs light and 12 hrs night cycle with a temperature between 22–25°C and humidity 60–70%). The animals were fed with standard pellet diet, provided by the same institution. The use and care of rats were done according to the guidelines for laboratory animals of the National Institutes of Health (NIH) [47]. The experimental protocol used in this study was approved by institutional animal ethical committee of the Department of Pharmacy, Southeast University.

2.7. Experimental Design

In the experiment, a total of 40 rats were used. The rats were divided into six groups and each group contains five rats as follows:

Group 1: Control: Only food and water were administered to rats (Con)

Group 2: Disease Control: Alloxan monohydrate 120 mg/kg b.w. was administered intraperitoneally to rats (Alx)

Group 3: Alloxan 120 mg/kg b.w.; i.p. + Plant extract 200 mg/kg b.w.; p.o. (Alx+ EEAR 200)

Group 4: Alloxan 120 mg/kg b.w.; i.p. + Plant extract 400 mg/kg b.w.; p.o. (Alx+ EEAR 400)

Group 5: Alloxan 120 mg/kg b.w.; i.p. + Fenofibrate 30 mg/kg b.w.; p.o. (Fen)

Group 6: Alloxan 120 mg/kg b.w.; i.p. + Plant extract 200 mg/kg b.w.; p.o. + Fenofibrate 30 mg/kg b.w.; p.o. (Alx+ EEAR 200 + Fen)

2.8. Induction of Diabetes

Alloxan monohydrate was dissolved in 0.9% saline and administered to rats (120 mg/kg b.w., i.p.) to induce diabetes in groups 2–7 by a single intra-peritoneal injection (i.p.) after fasting 16 hrs [48]. After 1 week, with noticeable hyperglycemia rats (blood sugar level higher than 11.5–13.5 mmol/L) were selected and used for this experiment. Measurements of plasma glucose levels were examined by glucometer using a blood sample from tail-vein of rats.

2.9. Lipid Profile and Hepatic Function Tests

After 14 days of the treatment period on 15^{th} day, the rats from all the experimental groups were sacrificed by using anesthesia (diethyl ether) to open their chest to obtain blood sample. By using heparinized syringes blood sample were withdrawn directly from aorta of heart and kept in test tube containing anticoagulant (EDTA). Ultra-centrifuge machine (Centurion, UK) was used to centrifuge blood samples at an rpm of 4000 for 20 min to separate serum. Then the serum was preserved at −20°C for investigating TC, TG, LDL-cholesterol, VLDL-cholesterol, HDL-cholesterol, SGOT, SGPT and TP concentrations by using UV spectrophotometric method (Shimadzu UV-1200, Tokyo, Japan) with the help of wet reagent diagnostic kits according to manufacturer’s protocol.

2.10. Measurement of Body Weight and Organ Weight

The body weight of rats of the entire experimental group had been determined on 0^{th}, 5^{th}, 10^{th} and 14^{th} day of the treatment period. After the sacrifice of the whole rat groups the liver, kidney, pancreases, heart, and lung were being detached and cleaning of the adjacent tissues had been brought about. Ultimately, measurement of organ weight was instantly being done. The ratio of organ weights to body weight ratio (O/B) were estimated and kept in 10% formalin, refrigerated at −20°C.

2.11. Statistical Analysis

Data were expressed as mean ± SEM. Statistical comparison was performed by one-way (ANOVA) followed by Bonferroni's multiple comparison test and the values were considered as statistically significant when p values were less than 0.05 ($p < 0.05$). Graph Pad Prism, Version-7 (GraphPad, Software, Inc. 7825 Fay Avenue, Suite 230La Jolla, CA 92037 USA) and Microsoft Excel 2010 (Roselle, IL, USA) were used for the statistical and graphical evaluations. The results were considered as statistical significance at $p < 0.05$ compared to disease control and fenofibrate treated groups.

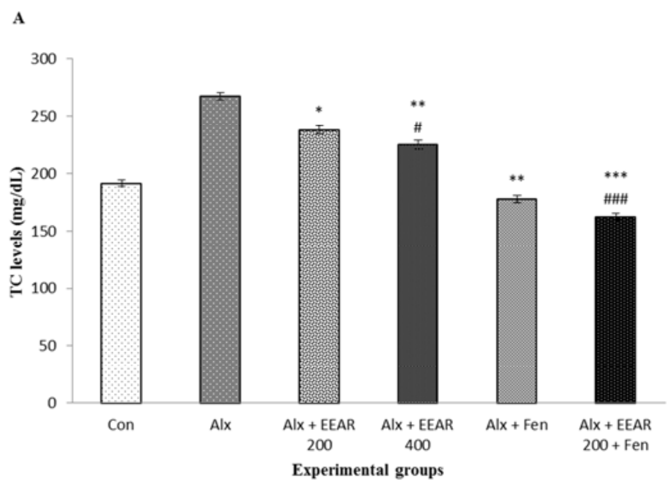
3. Result

3.1. Determination of Acute Oral Toxicity

EEAR up to the dose level of 2000 mg/kg b.w. had no adverse effect on behavioral, motor and neuronal responses of the rats during 14 days of observation. Different doses of EEAR displayed that there were no signs of alterations in the skin, eyes, fur and body weight thus the extracts were deliberated safe.

3.2. Effect of EEAR, Fenofibrate and Combination Therapy on Lipid Profile

The effect of EEAR, fenofibrate and the combination therapy on the lipid profile of rats is shown in Figure 1. The serum TG, TC, LDL and VLDL cholesterol levels were meaningfully greater in the disease control group, whereas the HDL cholesterol levels were noticeably decreased in the disease control rats. The administration of combination therapy significantly ($p < 0.001$; $p < 0.001$) ameliorated the activity of TC, TG, LDL, VLDL and HDL cholesterol levels when compared to that of disease control rats and fenofibrate treated rats. The ingestion of EEAR, markedly modified ($p < 0.05$, $p < 0.01$, $p < 0.001$; $p < 0.05$, $p < 0.01$) the activity of TC, TG, LDL, VLDL and HDL cholesterol levels of the rats in a dose-dependent mode when compared to the disease control rats and fenofibrate treated rats.



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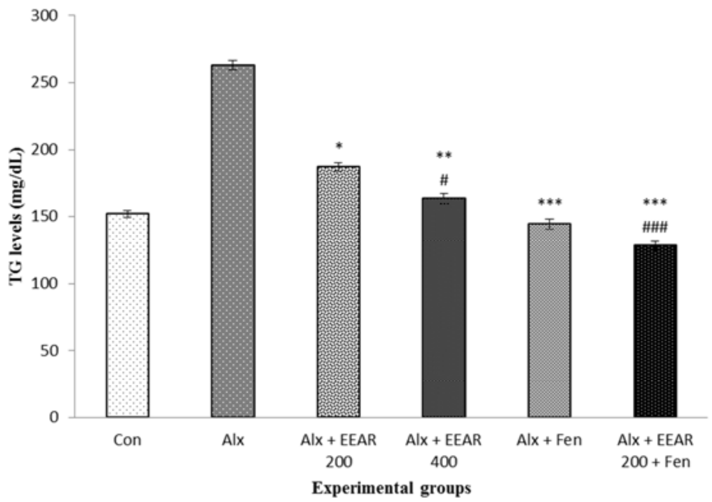
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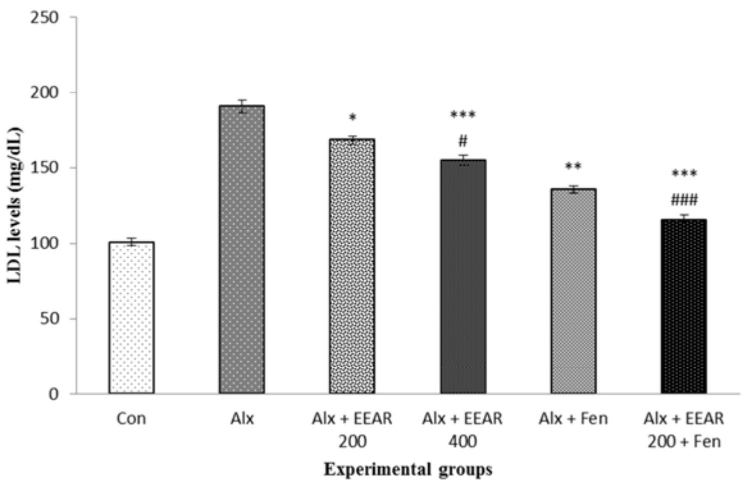
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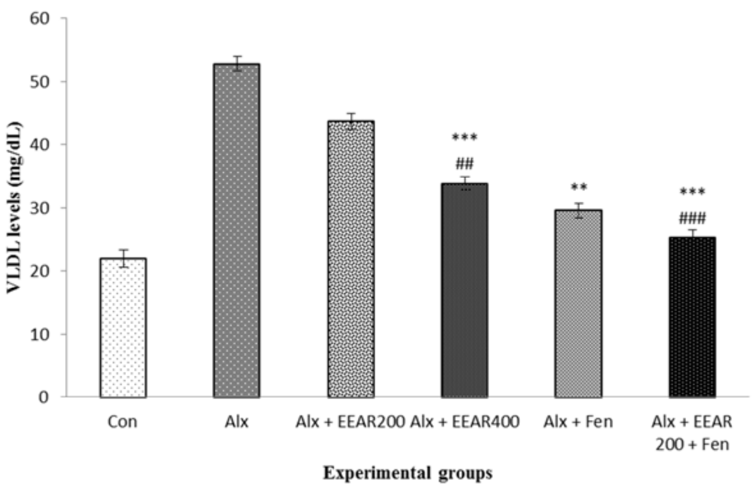
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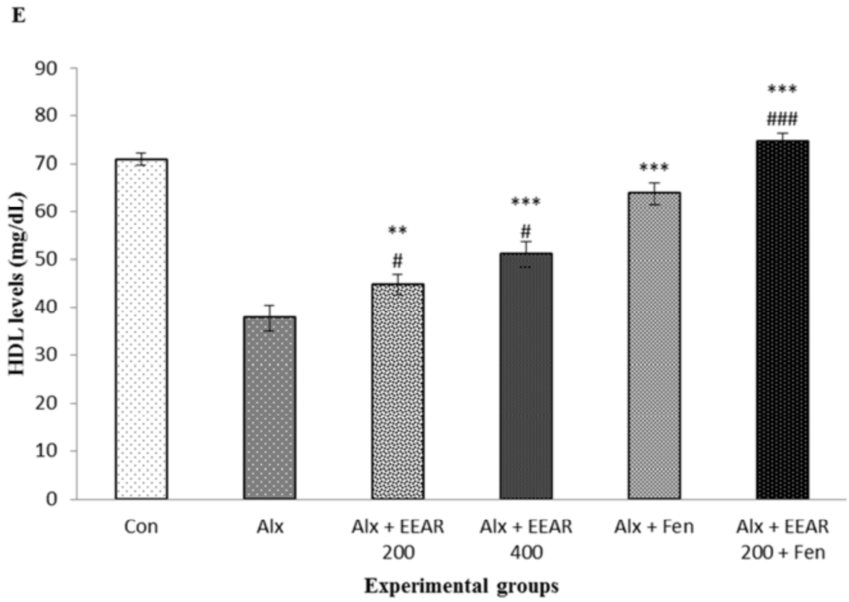
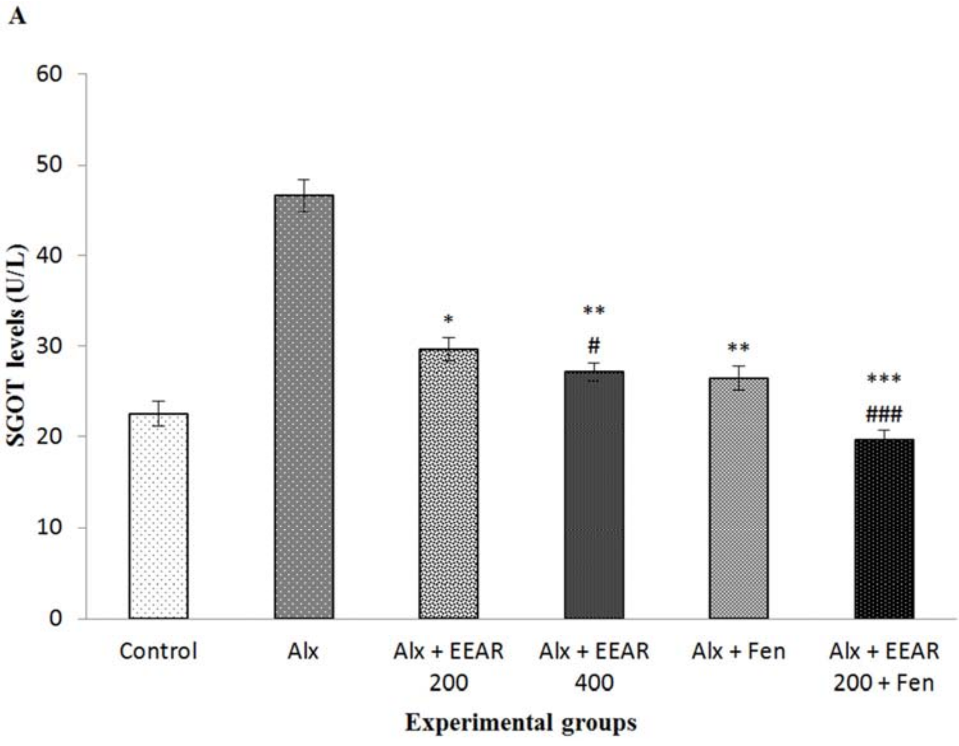
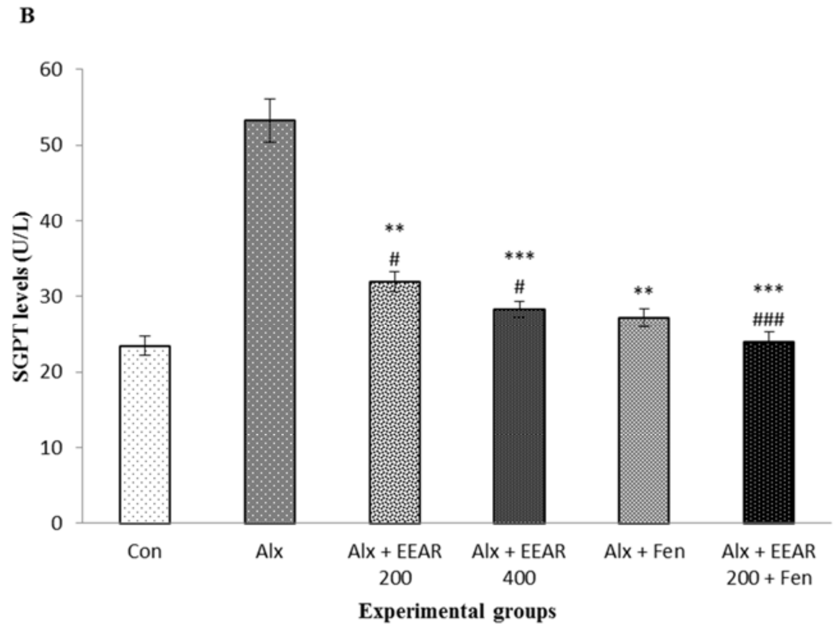


Figure 1. *Hypolidemic effect of EEAR, fenofibrate and combination therapy on lipid profile in diabetic rats. Values were expressed as mean ± SEM (n = 5/group). A. Total cholesterol; B. Triglycerides; C. Low density lipoprotein; D. Very low density lipoprotein; E. High density lipoprotein.* $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *significant difference from the disease control group.* $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ *significant difference from the fenofibrate treated group.*

3.3. Effect of EEAR, Fenofibrate and Combination Therapy on Hepatic Functions

The effect of EEAR, fenofibrate treatment and the combination therapy on the hepatic functions of rats is shown in Figure 2. The hepatic marker enzymes, including SGOT, SGPT and TP levels were noticeably higher in the disease control rats. The administration of EEAR pointedly ($p < 0.05$, $p < 0.01$, $p < 0.001$; $p < 0.05$) reduced the liver enzymes level, such as SGOT, SGPT and TP in a dose-dependent manner as compared to that of disease control rats and fenofibrate treated rats. The effect of combination therapy significantly ($p < 0.001$; $p < 0.001$) decreased the SGOT, SGPT and TP hepatic marker enzyme levels when compared to the disease control rats and fenofibrate treated rats indicated amelioration in liver dysfunctions.





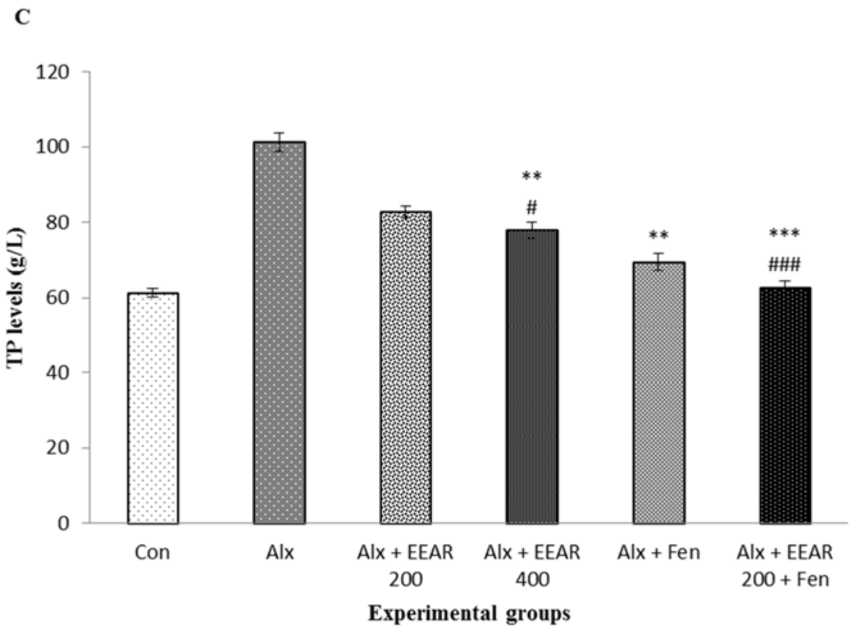


Figure 2. *Hepatoprotective effect of EEAR, fenofibrate and combination therapy on SGOT, SGPT and TP in diabetic rats. Values were expressed as mean ± SEM (n = 5/group). A. Serum glutamate oxaloacetate transaminases; B. Serum glutamate pyruvate transaminases; C. Total protein.* $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *significant difference from the disease control group.* $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ *significant difference from the fenofibrate treated group.*

3.4. Determination of Survival Rate

The survival rate of rats during 14 days of treatment in all the experimental groups is shown in Table 1. After 14 days of treatment, it was found that the survival rate among the EEAR treated rats were identical (Alx+ EEAR 200 = 60%, Alx+ EEAR 400 = 60%). The minimal survival rate was observed among the diabetic control rats (Alx = 40%) and fenofibrate treated rats (Fen = 40%) were also identical. The maximum survival rate was found in combination therapy treated rats (Alx+ EEAR 200 + Fen = 100%).

Table 1. *Survival rate of rats during 14 days of treatment with EEAR, fenofibrate and combination therapy.*

Groups	n			Survival
	Total Rats	Survivors	Deaths	(%)
Con	5	5	0	100
Alx	5	2	3	40
Alx + EEAR 200	5	3	2	60
Alx + EEAR 400	5	3	2	60
Fen	5	2	3	40
Alx + EEAR 200 + Fen	5	4	1	100

Values for survival rate of rats were expressed as percentages (n = 5/group).

3.5. Effect of EEAR and Combination Therapy on Body Weight and Organ Weight Changes

Body weight changes in all the experimental groups of rats are shown in Table 2. The body weight of disease control rats showed significantly decreased during 14 days of the treatment period. During treatment period, it was found that the significant ($p < 0.01$, $p < 0.001$; $p < 0.05$, $p < 0.01$, $p < 0.001$) changes in the body weight were observed in the EEAR and combination group on 10th and 14th day as compared to that of disease control rats and fenofibrate treated rats. The weight of heart, liver, lung, pancreas and kidney did not change considerably after 14 days of treatment in all the experimental groups of rats. Although the weight of liver and weight of pancreas significantly decreased in disease control group, after 14 days of treatment the values were normalized ($p < 0.001$; $p < 0.05$, $p < 0.01$, $p < 0.001$) in maximum doses of EEAR (Alx+ EEAR 400) and combination therapy treated rats when compared to that of disease control rats and fenofibrate treated rats.

Table 2. *Effect of EEAR, fenofibrate and combination therapy on body weight changes in diabetic rats.*

Groups	Body Weight Changes (g)			
	0th Day	5th Day	10th Day	14th Day
Con	153.93 ± 2.257	156.67 ± 1.405	159.24 ± 1.514	160.33 ± 0.544
Alx	141.18± 1.828	136.92 ± 2.441	132.36 ± 2.518	126.26 ± 1.941
Alx + EEAR 200	153.10 ± 1.815	150.10 ± 3.837	151.37 ± 2.202**#	152.49 ± 1.110***#
Alx + EEAR 400	147.59 ± 1.872	152.32 ± 1.775	153.92 ± 0.887***#	157.69 ± 1.111***##
Fen	146.85 ± 4.632	147.84 ± 2.989	150.02 ± 3.224**	153.03 ± 3.104***
Alx + EEAR 200 + Fen	145.19 ± 3.729	149.23 ± 2.274	150.80 ± 1.511***###	154.58 ± 3.625***###

Values were expressed as mean ± SEM (n = 5/group). **$p < 0.01$, ***$p < 0.001$ significant difference from the disease control group. #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ significant difference from the fenofibrate treated group.

Table 3. *Effect of EEAR, fenofibrate treatment and combination therapy on organ weight changes in diabetic rats.*

Groups	Organ Weight Changes (g)				
	Heart	Liver	Lung	Pancreas	Kidney
Con	0.57 ± 0.0318	4.71 ± ± 0.2908	0.49 ± 0.0219	1.38 ± 0.0318	1.07 ± 0.0706
Alx	0.51 ± 0.0203	3.38 ± 0.0498	0.45 ± 0.0416	0.99 ± 0.0410	1.09 ± 0.0706
Alx + EEAR 200	0.55 ± 0.0463	4.33 ± 0.0899	0.51 ± 0.0115	1.12 ± 0.0291	0.97± 0.0088
Alx + EEAR 400	0.57 ± 0.0285	4.38 ± 0.0273***##	0.41 ± 0.0949	1.26 ± 0.0321***#	1.01 ± 0.0145
Fen	0.55 ± 0.0318	5.29 ± 0.0536	0.47 ± 0.0203	1.14 ± 0.0145	1.09 ± 0.0643
Alx + EEAR 200 + Fen	0.49 ± 0.0203	5.54 ± 0.0491***###	0.47 ± 0.0208	1.32 ± 0.0153***###	0.99 ± 0.0088

Values were expressed as mean ± SEM (n = 5/group). ***$p < 0.001$ significant difference from the disease control group. #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ significant difference from the fenofibrate treated group.

4. Discussion

Dyslipidemia is one of the major risk factors for cardiovascular disease in diabetes mellitus [48, 49]. The relationship between diabetes mellitus and serum lipid profile had been much discussed during the past decades [50-52]. Both lipid profile and diabetes have been shown to be the important predictors for metabolic disturbances including dyslipidemia, hypertension, cardiovascular diseases, hyperinsulinemia, etc [53, 54].

In the present study, alloxan treatment showed a significant elevation in glucose and lipid levels i.e., TC, TG, LDL and VLDL and a reduction in HDL and also reported that elevation of hepatic markers i.e., SGOT, SGPT and TP levels. The possible mechanism might be that free radical generation by the alloxan causes damage to β-cells of pancreas, leading to insulin deficiency which results in hyperglycemia and also associated with hyperlipidemia [55]. The mechanism of alloxan induced hepatotoxicity could be attributed to decrease in antioxidant enzymes, accompanied by a significant increase in aldehyde products of lipid peroxidation, leading to hepatic oxidative stress in rats [56].

Lipids play an important role in the pathogenesis of diabetes mellitus [57]. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease [58]. Lowering of serum lipids levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease [59]. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase [60]. The elevated hypertriglyceridemia was increased in the synthesis of triglyceride rich lipoprotein particles, VLDL in liver diminished catabolism in diabetic rats [61]. Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver [62]. The increased levels of LDL and VLDL in the diabetic animals might be due to overproduction of LDL and VLDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx [63]. The HDL was significantly reduced in the diabetic rats, which indicate a positive risk factor for atherosclerosis [64]. In the present study, alloxan induced diabetic rats had an elevation in the serum lipids. The effect of EEAR markedly modified the activity of TC, TG, LDL, VLDL and HDL cholesterol levels when compared to the disease control rats and fenofibrate treated rats. The administration of combination therapy meaningfully improved the activity of TC, TG, LDL, VLDL and HDL cholesterol levels as compared to that of disease control rats and fenofibrate treated rats. In a previous study on lipid profile by Dheeba et al., also reported analogous findings for the roots extract of *Asparagus racemosus* in alloxan-induced diabetic rats [65].

The liver is an important insulin-dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected by diabetes [66]. The liver is one of the organs damaged by free radicals [67]. Several studies have shown that oxidative free radicals generated by alloxan administration being the most common etiology for the destruction of vital organs of the body [68, 69]. It was evident an increase in activities of the hepatic marker enzymes SGPT, SGOT and TP indicated that diabetes might be induced due to liver dysfunction [70]. Liver necrosis in alloxan-induced diabetic rats augmented in the activities of SGPT, SGOT and TP in plasma might be mainly due to the leakage of these enzymes from the liver cytosol into the bloodstream [71, 72]. In our study, the levels of hepatic enzymes marker such as SGOT, SGPT and TP levels were pointedly higher in the disease control rats. After 14 days of treatment our study observed that the rats treated with EEAR markedly reduced the liver enzymes level, including SGOT, SGPT and TP when compared to that of disease control and fenofibrate treated rats. The administration of combination therapy expressively decreased the SGOT, SGPT and TP hepatic marker enzyme levels when compared to that of disease control rats and fenofibrate treated rats disclosed amelioration in liver dysfunctions. Rahimi et al., in the study on *Carthamus tinctorius* oil in alloxan-induced diabetic rats claimed noticeably improvement in the hepatic dysfunction [73].

The current investigation found that none of the rats died in combination groups. The survival rate was expressively higher in combination groups as compared to that of disease control and fenofibrate treated rats. Rajendran et al., in the study on nuts demonstrated similar outcomes when administered in combinations with *Emblica officinalis* and honey [74].

In the present study alloxan induced diabetic rats had lower body weight and organ weight (heart, liver, lung, pancreas and kidney). The decrease in body weight could be due to an excess breakdown of tissue proteins [75]. Increased breakdown of glycogen and pronounced gluconeogenesis in diabetes might be responsible for the reduction in liver weight of diabetic animals [76]. Oral administration of combination therapy to diabetic rats pointedly improved liver weight and pancreas weight when compared to that of disease control rats and fenofibrate treated rats. In the study on the effect of the bark extract of *Ficus racemosa* in the body weight in alloxan-induced diabetic rats by Sophia et al., reported similar results [77].

5. Conclusion

Present study exhibited that common lipid abnormalities and liver dysfunction were observed in alloxan induced diabetic rats. Outcomes suggest a greater dominance of dyslipidemia, which might be playing a principal role in the progression of CVD among diabetic rats. From our experimental findings, it can be concluded that the effect of EEAR potentiates the activity of fenofibrate by increasing HDL level significantly, but reducing TC, TG, LDL, VLDL as well as SGOT, SGPT and TP level. It causes rapid induction of hypolipidemia as well as hepatoprotective effect in diabetic rats. In this study, EEAR showed a natural key in hypolipidemic and hepatoprotective activity. Further identification and isolation of active phytochemical constituents of AR and their fundamental mode of action accountable for hypolipidemic and hepatoprotective activity may be beneficial in developing a potent molecule for DM associated with CVD and hepatic complications.

Abbreviations

DM: Diabetes mellitus; T1D: Type 1 diabetes; T2D: Type 2 diabetes; CVD: Cardiovascular diseases; AR: *Asparagus racemosus*; EEAR: Ethanolic extract of Asparagus racemosus; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; WHO: World health organization; γGT: gamma-glutamil transferases; b.w.: Body weight; p.o.: Per os (by mouth); TC: Total cholesterol; TG: Triglycerides; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; HDL: High density lipoprotein. SGOT: Serum glutamate oxaloacetate transaminases; SGPT: Serum glutamate pyruvate transaminases; TP: Total protein; Con: Control; Alx: Alloxan; Alx + EEAR 200: Alloxan 120 mg/kg b.w., i.p. + Plant extract 200 mg/kg b. w., p.o.; Alx+ EEAR 400: Alloxan 120 mg/kg b.w., i.p. + Plant extract 400 mg/kg b.w., p.o.; Fen: Alloxan 120 mg/kg b.w., i.p. + Fenofibrate 30 mg/kg b.w., p.o.; Alx + EEAR 200 + Fen: Alloxan 120 mg/kg b.w., i.p. + Plant extract 200 mg/kg b.w., p.o. + Fenofibrate 30 mg/kg b.w., p.o.

Ethical Approval

The protocol of the experiment was approved by the animal ethics committee of the Department of Pharmacy, Southeast University, Dhaka, Bangladesh. The animals care and health were maintained according to the guidelines of NIH.

Author's Contributions

AAM: Designed the study, wrote the protocol and managed the analyses of the study and prepared the draft of the manuscript. AAM and MH: Carried out the laboratory tests. AI: Prepared the plant extracts and managed the literature searches. AAM: Performed statistical and graphical evaluations. MSU and SZ: Reviewed the scientific contents of the manuscript. All the authors read and approved the final manuscript.

Conflict of Interests

The authors proclaim that there is no conflict of interests exist about the content of this paper.

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