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RESEARCH ARTICLE

Diabetic rats: Hyperlipidemia and role of mango seed

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ABSTRACT

Background: Diabetes is a hyperglycemic disease caused by a lack of action of insulin, but serum lipids are also strongly affected by insulin. Serum lipid abnormalities (dyslipidemia) are frequently observed in diabetic populations regardless of insulin deficiency or insulin resistance. Hypertriglyceridemia is the greatest common serum lipid abnormality in diabetic populations. To study the effect of mango seeds on dyslipidemia in diabetes-induced rats. There was a noticeable difference in the body weight (BW) of male and female diabetic rats.

Materials and methods: To investigate effect on BW and the antihyperlipidemic actions of ethanolic extract of mango seed (EEMS) on alloxan-mediated diabetic Sprague-Dawley rats. Antihyperlipidemic in addition antidiabetic properties of an EEMS was planned at 100 and 200 mg/kg BW. The antidiabetic effect of EEMS been matched with tolbutamide 500 mg/kg BW. The lipid levels and BW of male and female rats were examined at consistent periods throughout the research.

Results: The outcomes displayed that the dyslipidemia was considerably decreased in the different treatment groups, while matched with control, the animals cured with tolbutamide and EEMS control.

Conclusion: EEMS exhibited a noteworthy antihyperlipidemic actions in addition with marked difference on BW of both male and female in alloxan-induced diabetic rats.

Keywords: alloxan-induced diabetes, ethanolic extract of mango seed, dyslipidemia, antihyperlipidemic, body weight

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INTRODUCTION

Diabetes mellitus is a syndrome with abnormal metabolism described by the high sugar, dyslipidemia or hyperlipidemia, and glycosuria, usually because of insulin opposition or insulin insufficiency [1].

Diabetic dyslipidemia is as the metabolic condition might evidence to the intensification of the primary cardiac illness [2]. The load of cardiac illness is intensifying each time and it is main reasons of illness and death predominantly great amongst patients having type 2 diabetes [3]. In diabetes, plasma lipid levels are exaggerated as of an association relating to simple carbohydrate insulin-resistance is a collective irregularity perceived in diabetes, which could upsurge very low-density lipoprotein (VLDL) discharge. In type 1 and type 2 diabetes, deprived glycemic mechanism upsurges serum triglyceride (TG), intermediate-density lipoproteins (IDL), and VLDL and falls high-density lipoprotein (HDL) cholesterol levels. It is expected that 30%-60% of patients of type 2 diabetes take dyslipidemia [4]. Freshly, here is great attention in the medicinal herb used for the treatment of diabetes as the oral hypoglycemic drugs will carry additional collective unwanted side effects such as minor hypoglycemia and gastrointestinal hitches [5]. Though, the medicine plants are extremely active and small rates as well as slighter reactions when matched to the oral hypoglycemic, thus they are recommended [6]. The antidiabetic action of the therapeutic plants has proven numerous processes, comprising renal glucose absorption, insulin degradative actions, inhibition and encouragement of beta cells of islets of Langerhans for insulin secretion, reduction the resistance of insulin, and

reinforcing or restoring the pancreatic beta cells by aggregating the extent and quantity of the cells in islets of Langerhans [7]. Mangifera indica (MI), too well-known as mango and well known as the king among tropical fruits cultivated in many regions of subcontinent. Mangoes belong to the genus Mangifera, consisting of about 30 species of tropical fruit plants in the family of anacardiaceous [8]. The chief components of mango seed are starch, fat, and protein. Mango seed comprise of about 44%-48% saturated fatty acids and 52%-56% stearic unsaturated fatty acids. Seed grains have little protein content, but then have maximum of the crucial amino acids, with higher standards of lysine, valine, and leucine [9]. It has been reported that the extracts have several therapeutic activities such as anti-inflammatory, anti-oxidant, anti-microbial activity, anti-parasitic, anti-helminthic, anti-allergic, anti-spasmodic, anti-cancerous, anti-viral, anti-diarrheal, also proven as treatment of hemorrhoid, hepatoprotective, and gastroprotective agent [10]. Diabetes induced dyslipidemia consequences of mango seed is not clear. Hence, the present study was intended to examine the antihyperlipidemic activity of ethanolic extract of mango seed (EEMS) on alloxan-induced diabetes in Sprague-Dawley rats.

MATERIALS AND METHODS

Sample Collection and Preparation of Kernel

The fresh ripe mangoes without bruises, weighing 200-250 grams stood bought from local bazaar. Pulp was evenhanded later fruit and seed were selectively detached and splashed by purified water to take away surface traces. Kernel core was detached as of stone skin, shack sun-drying for four times, crushed in an electrical chopper and exposed for removal by ethanol till 72 hours.

Preparation of Extract

The mango kernel fine particles was removed 80% ethanol, abstract acquired was clarified concluded Whatman No.1 filter paper, and remainder achieved was made intense below condensed compression consuming a spinner evaporator at 40°C and stayed lyophilized. The lyophilized residue keep on put in storage at 4°C till additional consumption [11]. The extract powder was ran in purified water and recycled for in vitro and in vivo research.

Experimental Animals

Healthy adult, Sprague-Dawley male and female rats, (female ensured to be non-pregnant weighing 160-175 grams) were used for antidiabetic and antihyperlipidemic studies. The rats acquired from the investigational animal breeding unit of animal house of Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, Karachi, ERC number F.1-2-/2020/BMSI-E COMT/080JPMC and stayed held in harmony using the principals for the precaution and usage of research laboratory animals of the organization. The rats remained in investigational confines below a 12 h light-dark cycle at an optimal temperature of 25±2°C, to adapt for seven days. Throughout the adjustment time, the rats existed on rodent chow and with access to water.

Experimental Design/Induction of Diabetes

It was an analytical randomized study and was piloted in Department of Pharmacology and Therapeutic, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Center, Karachi. The experimental protocol was designed for 21 days. The rats either male or female were randomized into five groups, with ten animals in each group (n=10).

Group A: Normal control (without diabetes).

Group B: Diabetic control (without treatment).

Group C: Diabetic rats cured with tolbutamide 500 mg/kg body weight (BW).

Group D: Diabetic rats cured with100 mg/kg ethanol extract of mango seed.

Group E: Diabetic rats cured with 200 mg/kg ethanol extract of mango seed.

Induction of diabetes mellitus: Single intra-peritoneal (IP) injection of 120 mg/kg BW of alloxan monohydrate (Sigma, St. Louis, USA) was administered in 18 h fasted animals to induce diabetes mellitus (DM). A fasting plasma glucose concentration (FPGC)>110 mg/dL in the rats for five successive days were reflected a diabetic state and designated for the experiment.

Body Weight Analysis

At consistent intermissions, body mass of investigational male and female animals were documented and modification in BW was designed.

Biochemical Analysis

On the conclusion of research, blood sample were obtained from the tail of animals and the serum remained separated by centrifuging on 3,000 RPM for 20 minutes. The serum samples were recycled for assessment of lipid parameters such total serum cholesterol (TC), TG, and HDL, and low-density lipoprotein (LDL) levels were assessed via commercially used standard kits (Randox Research Laboratory, Ltd., Crumlin, Co. Antrim, UK), agreeing to the company's guidelines.

RESULTS

Consequence of the tolbutamide, as well as EEMS on BWs of diabetic male and female rats were shown in **Table 1** and **Table 2**, respectively. Management of tolbutamide and EEMS to rats with amassed doses of 500 mg/kg, 100 mg/kg, and 200 mg/kg showed important modification in rats' BW.

Variations in BWs (g) of male rats were offered in **Table 1** showed that normal control had 4.28% BW increase, diabetic control underwent -1.89% decrease in BW; and rats treated with tolbutamide and EEMS (100 mg/dl and 200 mg/dl) had BW gain of 4.32%, 2.53%, and 9.43%.

Table 1. Variabilities in BW (g) of diabetic male rats before & after 21 days of treatment

Rat group	Preliminary weight	Concluding weight	Weight variation (%)
Normal control	155.80±4.09	164.40±4.12	4.28
Diabetic control	159.80±3.15	156.80±3.15	-1.89
Tolbutamide (500 mg/kg)	162.80±3.15	169.80±3.22	4.32
Ethanol extract of mango seed (100 mg/kg)	158.70±1.76	162.10±1.19	2.53
Ethanol extract of mango seed (200 mg/kg)	159.60±1.57	174.10±2.18	9.43

Table 2. Variabilities in BW (g) of diabetic female rats before & after 21 days of treatment

Rat group	Preliminary weight	Concluding weight	Weight variation (%)
Normal control	155.80±4.09	168.40±4.12	5.28
Diabetic control	159.80±3.15	156.80±3.15	-1.88
Tolbutamide (500 mg/kg)	162.80±3.15	172.80±3.22	6.17
Ethanol extract of mango seed (100 mg/kg)	158.70±1.76	162.10±1.19	2.50
Ethanol extract of mango seed (200 mg/kg)	159.60±1.57	176.10±2.18	10.60

Table 3. Effect of tolbutamide on total lipid profile of rats after 21 days

Lipid profile	Normal control	Diabetic control	Tolbutamide (500 mg/kg)	p-value
Triglycerides (mg dL-1)	79.20±7.20	129.70±9.10	80.47±4.13	0.05
Total cholesterol (mg dL-1)	124.40±8.90	305.30±15.70	152.40±1.50	0.05
LDL (mg dL-1)	43.80±6.40	206.00±18.30	67.70±1.15	0.05
HDL (mg dL-1)	55.40±5.20	38.90±4.30	49.80±3.22	0.05

Note. Levels differ significantly ($p<0.05$) & controls and diabetic rats treated with tolbutamide

Table 4. Effect of EEMS on lipid profile of rats subsequently 21 days management with different dilutions

Lipid profile	Normal control	Diabetic control	EEMS (100 mg/kg)	EEMS (200 mg/kg)	p-value
Triglycerides (mg dL-1)	79.20±7.20	129.70±9.10	98.60±6.30	84.90±5.70	0.05
Total cholesterol (mg dL-1)	124.40±8.90	305.30±15.70	164.80±12.40	159.60±13.20	0.05
LDL (mg dL-1)	43.80±6.40	206.00±18.30	121.40±11.60	73.80±8.20	0.05
HDL (mg dL-1)	55.40±5.20	38.90±4.30	46.40±2.80	49.10±4.80	0.05

Note. Levels differ significantly ($p<0.05$) & controls and diabetic rats treated with 100 mg/dl & 200 mg/dl EEMS

The alterations in BWs (g) of female rats were untaken in **Table 2** showed the normal control ensured 5.28% BW addition, diabetic control underwent -1.88% reduction of BW; and rats treated with tolbutamide and EEMS (100 mg/dl and 200 mg/dl) had BW gain of 6.17%, 2.50%, and 10.6%, respectively, as shown in in **Table 1** and **Table 2**. There is slight difference in weight gain of male and female.

Values represent mean [M]±standard deviation [SD]. Values change significantly ($p<0.05$). Controls and diabetic rats treated with tolbutamide, 100 mg/dl and 200 mg/dl EEMS (tests applied ANOVA as well as Tukey's post-hoc test).

Controls and diabetic rats treated with tolbutamide, 100 mg/dl and 200 mg/dl EEMS (tests applied ANOVA as well as Tukey's post-hoc test).

The properties of EEMS on the lipid profiles levels of the rats are presented in **Table 3** and **Table 4**. Comparative to the normal control, the diabetic rats had significant ($p<0.05$) rise in plasma total cholesterol, triglycerides, LDL, and VLDL; with a simultaneous significant ($p<0.05$) reduction in plasma HDL concentration. Conversely, in association with the diabetic control, group treated with tolbutamide had significantly ($p<0.05$) lower levels of plasma total cholesterol, triglycerides, LDL, and VLDL with an attendant significant ($p<0.05$) increase in HDL. Conversely, the group treated with EEMS had also showing significantly ($p<0.05$) lesser levels of plasma total cholesterol, triglycerides, LDL, and VLDL; with an associated significant ($p<0.05$) rise in HDL. The 200 mg/dl EEMS remained additional effective in refining the lipid profile of diabetic rats than 100 mg/dl EEMS as shown in then **Table 3** and **Table 4**.

STATISTICAL ANALYSIS

Statistical software SPSS version 21.0 was employed for data serving and exploration. A descriptive statistical investigation of continuous parameters were done. Quantitative variables were shown in term of mean and standard deviations. ANOVA and post-hoc-Tuckey test were used to relate mean as right $p \leq 0.05$ was measured.

DISCUSSION

In current study, the antihyperlipidemic impact of the EEMS was planned against the alloxan-induced diabetic rats. There was great impact of gender on experimental diabetic rats. The male rats were more subtle to the induction of diabetes and noteworthy alterations in BW between groups were detected.

The rats in control and tolbutamide cured group revealed gradual rises in BW and this might be owing to the development of the skeletal dimension, epididymal fat and the increases of body mass index. There existed an important variation in BW of both male and female rats.

Serum lipid abnormalities (dyslipidemia) are usually perceived in diabetic people regardless of insulin insufficiency or insulin opposition [12]. It is main threat for cardiac disease (CVD) in DM [13]. Irregularities in lipid digestion ensuing after raised free fatty acid discharge from insulin-resistant fat cells clues to modifications in plasma lipid profile in diabetes [14].

Perversely, the lipoprotein lipase exists deactivated below situation of insulin absence and/or confrontation, leading to hypertriglyceridemia. The modifications in the plasma lipid profile of diabetic rats in this research be present in clearance through the changes in lipid profiles of HFD-STZ- induced diabetic rats described by other investigators [15]. Raised LDL level increases the accumulation of cholesterol in the arteries and aorta and HDL is considered as an advantageous lipoprotein that inhibits cholesterol deposition, thereby inhibiting atherosclerosis [16].

According to our study, there were detected the reduction in the plasma triglyceride, total cholesterol, LDL, and an associated rise in the HDL levels of diabetic rats on EEMS may be owing to advanced insulin release through islet cells of the pancreas. There are certain natural remedies or products which also be used to increase insulin production. Mango is a fruit that has many benefits medically all of its parts are used in different types of treatments mango extract used nowadays for the treatment of diabetes. The extract concentrate may ensure both sort of diabetes and its entanglement by diminishing the glucose discharge by restraint of sugar hydrolyzing compounds and its retention during digestion, through increasing insulin and antioxidants, by decreasing cholesterol.

Tolbutamide is a kind of drug widely used among patients of diabetes; this medicine is classified in the sulfonylurea's group of drugs also used in previous studies to treat diabetes [17]. Tolbutamide helps in the production of insulin (a hormone that helps to maintain blood sugar levels by converting extra sugar into glycogen which is stored in the body) and supports to retain the balance of sugar in body. Along benefits this drug also has some side effects like gaining weight, increasing cholesterol, loss of appetite, etc.

To our understanding, this is the innovative research that examined the outcome of mango seed on lipid profile on animal model. Subsequently 21st days treatment, rats in the EEMS group had lower ($p<0.05$) total cholesterol, triacylglycerol, and low-density lipoprotein ($p<0.05$), HDL concentration matched to rats in the other groups coincided with other findings [18, 19] showing altered cholesterol and triacylglycerol.

In this research, we evaluated the anti-hyperlipidemic action of EEMS in diabetic rats. This finding is in conformity with finding of hyperlipidemic state of the diabetic rats. This observation suggests that the EEMS can reduction of lipid profile of diabetic rats. This study showed that EEMS applied an important dose dependent reduction in TAG, CHOL, LDL, and significant dose dependent rise in HDL level. Compared to other groups rats in the Extract group had advanced ($p<0.05$) HDL: LDL ratio but lower ($p<0.05$) LDL: HDL.

CONCLUSION

On the bases of the results obtained from the present study, it can be proposed that fruit waste as mango seed after some processing can be a good remedy against diabetes induced hyperlipidemia. Particularly mango peels showed promising effects against increased lipid profile Therefore, further research using mango seed for human subjects suffering from hyperlipidemia is recommended.

Author contributions: **AA:** conceived & designed study, conducted research, provided research materials, & collected & organized data; **FA:** analyzed & interpreted data; **FR:** wrote initial & final draft; **FA:** write up & review; & **MUF:** analysis & editing. All authors have agreed with the results and conclusions.

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Declaration of interest: No conflict of interest is declared by authors.

Data sharing statement: Data supporting the findings and conclusions are available upon request from the corresponding author.

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