Diabetic Dyslipidaemia and Hypolipidaemic Effect of Turmeric in Alloxan-Induced Diabetic Rats Original Research Article

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ABSTRACT

OBJECTIVES: To determine serum lipid lowering effect of turmeric in alloxan-induced diabetic rats.
STUDY DESIGN: A Randomized controlled trial (experimental animal study)
PLACE AND DURATION: Conducted at Postgraduate Medical Institute Lahore. Approval from ethical committee of animal sciences of PGMI was obtained before the start of experiment. The study was conducted from 1st September to 30th December, 2014, after induction of diabetes.
METHODOLOGY: In this study alloxan (intraperitoneal, 150mg/kg body weight) was used to produce animal models of type-I diabetes. The study was conducted on forty five albino rats. Animals were divided into three groups with fifteen animals each (normal control group A, diabetic untreated group B and diabetic group C treated with turmeric powder). Turmeric was given daily orally through 5cc disposable syringe (oral gavage method)in a dosage of 300mg/kg body-wt. dissolved in 4 ml distilled water per rat to all the 15 group C diabetic albino rats following one week after induction of diabetes. Blood samples of animals were taken from the saphenous vein after overnight fasting at zero day (one week following induction of diabetes in groups B and C), by the end of 8th week and by the end of 12th week respectively. Serum lipid profile was measured by direct quantitative method by using the commercially available Human kits. Analysis of data was done by using SPSS version 18.0.
RESULTS: Significantly elevated serum lipid levels (diabetic dyslipidaemia) were found in diabetic animals of group B and C after alloxan administration. Administration of turmeric for 12 weeks to group C diabetic animals showed significant improvements in serum lipid levels towards normal but not completely.
CONCLUSION: Diabetes induces significant alterations in serum lipid profile. Turmeric powder possesses hypolipidaemic effects.
KEY WORDS: Diabetes Mellitus, Diabetic Dyslipidaemia, Alloxan, Turmeric

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INTRODUCTION

Diabetes mellitus is a cluster of metabolic problems giving out the general characteristics of hyperglycaemia ensuing from absolute or relative deficiency of insulin[1]. Presence of chronic undiagnosed hyperglycaemia predisposes the subjects at larger risks of having microvascular and macrovascular problems[2].

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According to 2012 update of Diabetes Atlas more than 371 million community over the world is suffering from diabetes and half of the people do not know that they are having the disease[3].

There are profound alterations in plasma lipid profile associated with diabetes leading to high possibility of acute coronary syndrome and cerebral vascular accidents so after a proper diagnosis there should be early, effective and safe management of diabetes and its associated dyslipidaemia[4].

Diabetic dyslipidaemia is described as having hypertriglyceridemia, decreased values of HDL-c, postprandial lipemia and increased values of LDL-c[5]. The pathogenesis of diabetic dyslipidaemia is based mainly on lack of insulin and inability to respond to available insulin. In both the cases, the discharge of free fatty acids from adipose cells is increased leading to higher influx of these fatty acids into the liver. In liver if glycogen is sufficient, along with fatty acids there is increased production of triglycerides[6]. These higher levels of triglycerides lead to increased secretion of apolipoprotein B and VLDL-c leading to enhanced hepatic fat production and accumulation[7].

Alloxan was synthesized in 1838 as a pyrimidine derivative and was found to have a precise effect to damage the pancreatic beta cells in experimental animals[8]. Alloxan diabetes correlates with human diabetes type-I with final outcome of hyperglycae-

mia and insulinopenia[9]. Typical routes for alloxan administration are intravenous, intraperitoneal or subcutaneous[10].

Alloxan is more effective when given to overnight fasting animals[11]. Alloxan decomposes at neutral pH and body temperature. Its chemical nature is water soluble. Its half-life is 1.5 minutes. Its shape is similar to that of glucose[8]. Medicinal properties of herbs have stood through test of time predominantly for the cure of allergic, metabolic and degenerative problems. In Asian countries turmeric has widely been used as a spice, food additive and coloring material [12].

Turmeric (Curcuma Longa) plant is perennial, widely grown in humid climate. Botanically related to ginger family and has yellow colored rhizomes. It has been determined to exhibit anti-inflammatory, anti-diabetic and hypolipidaemic properties[13]. Turmeric powder contains curcuminoids, protein, fat, minerals (manganese, iron, and potassium), vitamin B6, dietary fiber and essential oils. Curcuminoids are polyphenols having anti-oxidant properties and have been identified as active principles of turmeric[14].

Turmeric has been used in clinical experiments to control both diabetes and its associated dyslipidaemia. Turmeric can improve lipid profile by altering the rate of cholesterol absorption, its breakdown or its removal from body[15]. Turmeric increases the production of adiponectin and reduces the oxidation of LDL so preventing the risk of atherosclerosis[16]. It can also serve as hypolipidaemic agent by acting as a ligand for PPAR and then activating it[17]. Rational of this experiment was to find out a harmless and cost effective remedy for metabolic derangements caused by diabetes. This study was conducted to determine the lipid lowering effect of turmeric in diabetic animals.

METHODOLOGY

This experimental animal study (randomized controlled trial) was conducted at Physiology department of PGMI (Postgraduate Medical Institute) Lahore. Approval from ethical committee of animal sciences of PGMI was obtained before start of experiment. It was approved by Advanced Science and Research Board of the University of Health Sciences (UHS), Lahore. The study was conducted from 1st September to 30th December, 2014, after induction of diabetes. 45 albino rats (both sexes), weighing 150-250 gm with average age of eleven weeks were selected for this study by simple random sampling using balloting method. Acclimatization of animals was done. The animals had daily basis adequate food provision and water ad libitum. The animals were kept separately in iron cages with optimum temperature (24±2 ^{0}C) and hygienic conditions.

Albino rats were taken from the animal house of Punjab University (department of Biological Sciences), Lahore. Alloxan vial was obtained from Merck Marker Lahore, Pakistan. Rhizomes of Curcuma Longa plant (Turmeric) were purchased from herbal store in local market.

Animals were divided into three groups with 15 animals each: control group A, diabetic group B and turmeric treated diabetic group C. After 12 hours fasting period, except control group A, animals were given alloxan intraperitoneally 150mg/kg body weight in 0.9% NaCl infusion single dose to induce diabetes. Diabetes was confirmed after five days by using glucometer. Inclusion criteria for animals in groups B and C was, having post prandial blood glucose level of ≥200 mg/dl and average weight between 150-250 gm. Animals of groups B and C with (post prandial blood glucose <200 mg/dl) were excluded from the experiment.

Roots of turmeric plant were washed, boiled and air dried for two weeks. Further dried in an incubator at 40 ^{0} C and were powdered in an electric grinder. The prepared powder was kept in clean, air tight glass bottle. It was administered daily orally through 5cc disposable syringe (oral gavage method)[18]in a dosage of 300mg/kg body-wt dissolved in 4 ml distilled water per rat to all the 15 group C diabetic albino rats following one week after induction of diabetes (day zero).

Blood samples (3 ml) were drawn from saphenous veins of overnight fasted animals of all the three groups and then directly transferred into a micro test tube for subsequent serum lipid profile estimation. Blood was centrifuged and serum was separated. Blood samples were collected at the start of experiment that is one week after induction of diabetes (day zero) which was the baseline for future evaluation, by the end of 8th week and by the end of 12th week respectively.

Serum triglycerides, LDL-c and HDL-c were estimated by enzymatic colorimetric method using commercially available pre-prepared Human kits. The estimations were made with an Automated Chemistry Analyzer Micro Lab LX 300.

Data analysis: The collected data was entered and analyzed using SPSS version 18.0. Serum lipid profile levels (quantitative data) were expressed in terms of mean and standard deviation (mean±SD). One way ANOVA (analysis of variance) was applied to find out mean differences among groups. A p-value of ≤ 0.05 was considered as statistically significant**.**

RESULTS

At baseline the mean serum triacylglycerol (TAG) level recorded for rats in healthy control group was 70±10 mg/dl, which remained in normal range during twelve weeks and was 66±10 mg/dl at the end. The serum triacylglycerol level for diabetic rats in group B and C increased to 138±24 mg/dl and 126±17 mg/dl after induction of diabetes. The serum triacylglycerol level of rats of group C declined gradually towards normal during the experiment with mean value 106±16 mg/dl at 8th week and 77±13 mg/dl at 12th week after treatment with turmeric. An increase in serum TAG level for rats in group B (diabetic) was recorded with mean serum triglyceride level 244±46 mg/dl at 8th week and 330±61 mg/dl at 12th week as shown in table - I.

Changes in serum LDL-c level of normal, diabetic and treated animals for the total duration of experiment are described in table - II. At baseline the mean serum LDL-c level recorded for animals in healthy control group was 80±10 mg/dl, which remained in normal range during twelve weeks and was 69±10 mg/dl at the end. The serum LDL-c level of diabetic animals in group B and C increased to 106±12 mg/dl and 126±21 mg/dl respectively after induction of diabetes. The serum LDL-c level of group C turmeric treated animals showed a decline during the experiment with mean of 97±21 mg/dl at 8th week and 67±16 mg/dl at 12th week. An increase in serum LDL-c level of animals in group B (diabetic) was recorded with a mean serum

LDL-c level 144±23 mg/dl at 8th week and 213±25 mg/dl at 12th week.

At baseline the average serum HDL-c level recorded for animals in healthy control group A was 29±4 mg/dl, which remained unchanged during twelve weeks. The serum HDL-c level for animals in diabetic groups B and C decreased to 19±4 mg/dl and 20±7 mg/dl respectively after induction of diabetes. The serum HDL-c level of animals decreased for group B during the experiment with mean of 15±5 mg/dl at 8th week and 12±4 mg/dl at 12th week. An increase in serum HDL-c level for animals in turmeric treated diabetic group C was recorded with a mean serum HDL-c level 24±8 mg/dl at 8th week and 28±7 mg/dl at 12th week (table - III).

trolled diabetic patients and chances of having atherosclerosis are obvious[19].

Dyslipidaemia in diabetic patients is attributed mainly to insulin resistance and insulin deficiency and lipid profile of type-I diabetic patients is highly dependent upon glycemic control[20]. In the current study, we determined the improvements in altered lipid profile with the use of turmeric powder in alloxan-induced diabetic rats.

A significant rise in serum triacylglycerol levels of animals in group B and C was observed following diabetes induction. This increase in serum TAG level of group B animals persisted throughout the experimental period. But a significant decline was seen in serum triacylglycerol levels in group C animals af-

Table - I: Serum TAG (mg/dl) levels in groups (A, B & C) at three reading times after induction of diabetes, (N= 45)

Groups	Baseline (day zero) Mean ± SD	8th Week Mean ± SD	12th Week Mean ± SD	p-value
Group A n=15	70 ± 10	71 ± 12	66 ± 10	0.488†
Group B n=15	138 ± 24	244 ± 46	330 ± 61	0.001***
Group C n=15	126 ± 17	106 ± 16	77 ± 13	0.001***

Results are expressed as mean±SD

Table - II: Serum LDL-cholesterol (mg/dl) levels in groups (A, B & C) at three reading times after induction of diabetes, (N= 45)

Groups	Baseline (day zero) Mean ± SD	8th Week Mean ± SD	12th Week Mean ± SD	p-value
Group A n=15	80 ± 10	73 ± 10	69 ± 10	0.001***
Group B n=15	106 ± 12	144 ± 23	213 ± 25	0.001***
Group C n=15	126 ± 21	97 ± 21	67 ± 16	0.001***

Results are expressed as mean±SD

Table - III: Serum HDL-cholesterol levels (mg/dl) in groups (A, B & C) at three reading times after induction of diabetes, (N= 45)

Groups	Baseline (day zero) Mean ± SD	8th Week Mean ± SD	12th Week Mean ± SD	p-value
Group A n=15	29 ± 4	29 ± 5	29 ± 4	0.949†
Group B n=15	19 ± 4	15 ± 5	12 ± 4	0.001***
Group C n=15	20 ± 7	24 ± 8	28 ± 7	0.002**

Results are expressed as mean±SD

DISCUSSION

Diabetic population is exposed to high dangers of having cardiovascular, peripheral vascular and cerebrovascular disease. Morbid conditions and premature mortality in diabetic cases often are the outcomes of various cardiovascular problems[5,18]. Alteration of plasma lipid profile is a common feature in uncon-

ter 12 weeks of treatment with turmeric powder.

In 2005, American Diabetes Association reported deficiency of insulin as the main culprit for increased free fatty acids flux to liver, leading to increased production of triacylglycerols and other cholesterol containing lipoproteins in diabetic patients [21, 22].Another study proposed that turmeric powder (curcumin) lowers triacylglycerol levels through provocation of multiple

fatty acid breakdown (TAG hydrolysis) and consumption (beta oxidation) pathways [23].

Our study revealed significantly elevated levels of LDL-c in group B and C diabetic animals one week after induction of diabetes. Group B diabetic untreated animals were found to have a significant, continuous rise in levels of serum LDL-c throughout the duration of study. Our findings are in harmony with different experimental trials in which serum lipid profile of alloxanized diabetic rats showed a significant increment in serum total LDL-c and VLDL-c levels along with TAG [21, 22].

Diabetic animals in group C were found to have a gradual decline in LDL-c after 12 weeks turmeric treatment as suggested by another study that both short term and long term dietary intake of turmeric powder could prevent dyslipidaemia with specific effect on serum LDL-c levels and LDL/HDL ratio. Lowering of LDL-c levels by turmeric may be produced through an increase in LDL receptors in liver tissue, thus providing a convenient path for LDL clearance from the body [24].

Many compositional abnormalities of lipoproteins like high concentrations of TG, LDL-c and low concentrations of HDL-c have been found in diabetic patients which predispose diabetics to increased risk of atherosclerotic heart disease[25]. Our current study showed significantly lower levels of HDL-c in group B and C diabetic animals at day zero. Continuous significant decrement in HDL-c levels of untreated diabetic animals of group B was noticed throughout the duration of study.

This study also revealed a positive trend in HDL-c levels as an increase occurred in group C turmeric treated animals. Another study on patients of metabolic syndrome with the use of herbal tea containing curcumin (active ingredient of turmeric) also documented improvements in serum lipid profile (TC, TG, LDL-c and HDL-c)[26]. This study emphasized on radical scavenging (antioxidant) activity of curcumin.

Some other trials revealed results which are contrary to our study. Probable reasons of statistically non-significant results could be due to small number of animals used, problems with absorption of turmeric through gastrointestinal tract and its low efficacy in advance stages of diabetes[27].

CONCLUSION

Diabetes induces significant alterations in serum lipid profile. Turmeric powder possesses hypolipidaemic effects.

CONTRIBUTION OF AUTHORS

Latif J: Conceived Idea, Designed Research Methodology, Manuscript Writing

Mukhtar S: Data Collection, Manuscript final reading and approval

Niaz S: Literature Search, Data Interpretation, Statistical Analysis

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