ORIGINAL ARTICLE

Hypoglycaemic Effect of Turmeric in Alloxan-Induced Diabetic Rats

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Abstract:

Background: Diabetes Mellitus is a syndrome of persistent hyperglycaemia, caused by absolute or relative deficiency of insulin. Diabetes is an advancing disease if uncontrolled and can lead to various macrovascular and microvascular compli-cations. Alloxan, a pyrimidine derivative is a potent diabetogenic agent used to develop type-I diabetes in animal models. Turmeric (Curcuma Longa) is a medicinal plant having antioxidant properties.

Objective: To determine hypoglycaemic effect of turmeric in diabetic rats and to compare serum glucose levels among groups.

Methodology: In this study alloxan (intraperito-neal, 150mg/kg body weight) was used to produce animal models of type-I diabetes. An experimental animal 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 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. Commercially available Human kits were used to find out serum glucose levels by glucose oxidase method. Analysis of data was done by using SPSS version 18.0.

Results: Significantly elevated levels of serum glucose were found after alloxan administration. Administration of turmeric in dosage of 300mg/kg body weight for 12 weeks to group C diabetic animals showed significant improvements in serum glucose levels towards normal.

Conclusion: Turmeric powder possesses hypoglycemic effects.

Key words: *diabetes mellitus, alloxan, turmeric*

Introduction

Diabetes mellitus is defined as clinical syndrome of chronically elevated blood glucose levels along with altered metabolism of carbohydrates, lipids and proteins due to errors of insulin release and insulin action1. Diabetes is classified into two broad categories: Type-I diabetes mellitus called insulin dependent diabetes (IDDM), is characterized by absolute deficiency of insulin release[2]. Type-II diabetes mellitus known as non-insulin dependent diabetes (NIDDM) is mainly the outcome of insulin resistance[3].

According to WHO criteria, fasting blood glucose level of a diabetic person should be equal to or > 126mg/dl on two occasions or > 200mg/dl two hours post glucose load[4]. 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

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disease[5].

The number of diabetics has reached to pandemic extent over the world and is escalating swiftly. In the estimates for diabetics in year 2000, Pakistan stood at number 6 with 5.2 million known diabetics and is expected to be at number 5 by year 2030 with 13.9 million diabetics[6].

Both environmental factors and heredity play their role in pathogenesis of diabetes[7]. In Asian region enhancement of number of diabetic patients is due to population overgrowth, aging, unbalanced diet and inactive life style[8].

There is association of glycaemia with complications of diabetes[9]. Higher insulin resistance is directly related to augmented possibility of having microvascular and macrovascular dangers of diabetes[10]. Most common of diabetic complications are diabetic foot amputations in developing countries[11]. In diabetic people major cause of morbidity is ischemic heart disease and altered lipid profile[12].

Alloxan is now known as potent diabetogenic agent to develop type-I diabetes in animal models[13]. 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[14]. Alloxan diabetes correlates with human diabetes type-I with final outcome of hyperglycaemia and insulinopenia[15]. Typical routes for alloxan administration are intravenous, intraperitoneal or subcutaneous[16].

Alloxan is more effective when given to overnight fasting animals[17]. 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[14].

From ancient times herbs and plant extracts are in common use of human being for treatment of various ailments. Medicinal properties of herbs have stood through test of time predominantly for the cure of allergic, metabolic and degenerative diseases[18].

Turmeric (Curcuma Longa) is a medicinal plant related to ginger (Zingiberaceae) family. Its plant is perennial with a small stem, large rectangle leaves and yellow colored rhizomes. This plant grows in countries with humid climate like India and China[19].

In Asian countries turmeric has been widely used as a spice, food additive and coloring material[20]. Turmeric powder contains Curcuminoids which are polyphenols with aromatic ring and have anti-oxidant properties[21]. Lack of toxicity of turmeric in mice, rats and even in some human trials is being documented[22].

Turmeric has been determined by various researchers to have a broad range of healing properties as anti-inflammatory, anti-tumor, anti-rheumatic, antidiabetic, hypolipidaemic, hypotensive, anti-ulcer and anti-oxidant[23]. Curcuminoids of turmeric possess anti-oxidant and hypolipidaemic properties, so protecting the beta cells of pancreas from burst of oxygen derived free radicals and improving their function[24]. In different studies turmeric was found to have effects of increased insulin production, enhancement of anti-oxidant enzyme activities and decreased lipid peroxidation[25].

Turmeric has been used in clinical trials for control of both type-I and type-II diabetes. Turmeric ingestion helps to up-regulate genes involved in breakdown of glucose while at the same time it is down-regulating the expression of genes with glucose synthesizing effect[26]. Turmeric can also improve insulin sensitivity by acting as ligand for PPAR-gamma and then stimulating the adipocyte differentiation[27].

Methods

This experimental animal study (randomized controlled trial) was conducted at Physiology department of PGMI 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. Duration of study was 12 weeks after induction of diabetes. 45 albino rats (both sexes), weighing 150-250 gm with average age of eleven weeks were selected. Acclimatization of animals was done. The animals hadadequate food provision and water ad libitum. The animals were kept separately in iron cages with optimum temperature (24±20C) 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 glucometer16. In group B and C, animals which demonstrated post prandial (random) blood glucose levels of ≥200 mg/dl were included in the experiment.

Roots of turmeric plant were washed, boiled and air dried for two weeks. Further dried in an incubator at 40°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)[28] 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 glucose estimation. Blood was centrifuged and serum was separated. Blood samples were collected at the start of experiment, 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.

Glucose oxidase (GOD-PAP) method was used for estimation of serum glucose levels by the use of pre-prepared commercially available Human kits. The collected data were entered and analyzed using SPSS version 18.0.The groups were compared by ANOVA and p value less than 0.005 is reported as significant.

Results

Two rats labeled no. 7 and 12 in group B (diabetic), expired three days before completion of 12th week experimental period.

At baseline the mean serum glucose level determined for rats in healthy control group was 92±15 mg/dl, which remained in normal range during twelve week time period and was 81±12 mg/dl at the end. The serum glucose level for rats in group B and C increased to 241±20 mg/dl and 239±28 mg/dl after induction of diabetes. The serum glucose level of animals declined for group C during the experiment with mean serum glucose level 195±27 mg/dl at 8th week and 150±24 mg/dl at 12th week. Serum glucose level of rats in group B was found to be high with mean serum glucose level 358±33 mg/dl at 12th week as depicted in table.

When comparison made among the groups at three reading times by applying one way ANOVA it was recorded that the mean serum glucose levels were significantly different with p-values <0.001 for baseline, 8th week and 12th week.

The pair-wise comparison by the use of Post hoc Tukey test explained a higher mean value of serum glucose in group B and C as compared to group A, both with p-values <0.001, and the difference between group B and C was non-significant with p-value 0.974 at baseline. At 8th week the group B and C had significantly higher average of serum glucose levels, by 202.9 mg/dl and 105.9 mg/dl both with p-values <0.001 as compared to group A and the difference between the group B and C was also significant with p-value <0.001. The mean serum glucose level of animals in group B increased to 276.9mg/dl by the end of 12th week as compared to group A with p-value <0.001 and was significantly higher by 208.0 mg/dl from group C with p-value <0.001. The difference of 68.9 mg/dl between group A and C was also significant with p-value <0.001.

Discussion

Derivation of newer, cheaper and safer herbal-based formulation which can bring the metabolic derangements of clinical diabetes towards near normal values was the ultimate purpose of our study. In this study we determined the improvements in serum glucose levels with the use of turmeric powder in alloxan-induced diabetic rats.

In comparison to animals of group A (control group), our study revealed significantly increased serum glucose concentration >200 mg/dl in diabetic animals of group B and C following induction of

Table: *Serum glucose (mg/dl) levels in groups (A, B & C) at three reading times after induction of diabetes where n= No. of rats in each group*

Groups	Baseline (day zero)	8th Week	12th Week	p-value
	mean±SD	mean±SD	mean±SD	
Group A n=15	92 ± 15	89 ± 15	81 ± 12	0.063 †
Group B n=15	241 ± 20[a]	292 ± 29[b]	358 ± 33[c]	0.001***
Group C n=15	239 ± 28[a]	195 ± 27[b*]	150 ± 24[c*]	0.001***

Group A = normal (control) group
Group B = diabetic group
Group C = diabetic treated group
Day zero = 1 week after alloxan injection
8th week = 8 weeks after induction of diabetes mellitus
12th week= 12 weeks after induction of diabetes mellitus
***** = very highly significant**
† = non-significant
a= significantly different from group A at day zero
b = significantly different from group A at 8th week
b* = significantly different from group A & B at 8th week
c = significantly different from group A at 12th week
c* = significantly different from group A & B at 12th week

diabetes with alloxan. Our results were in accord with findings of various other researchers who observed significantly raised blood glucose levels in rats following alloxan treatment due to lesion and necrosis of pancreatic beta cells and insulin deficiency[29,30].

Administration of turmeric powder to group C animals for 12 weeks produced statistically significant decrease in serum glucose concentration towards normal values when compared to diabetic untreated group B, which is in accordance with the results reported in different studies[25,26,31]. These trials reveal that turmeric powder could protect beta cells from oxidative stress of diabetes with specific effects on glucose utilization and insulin production.

Thus turmeric can have three effects. First is increased insulin production. Second is enhancement of antioxidant enzyme activities like catalase, super-oxide dismutase and glutathione peroxidase and third effect of turmeric is decreased lipid peroxidation with additional benefit of preventing the cardiovascular complications in diabetic patients[24,32].

However, the serum glucose concentration of turmeric treated diabetic animals of group C was still significantly higher than that of the control group A at the end of 12th week.

Conclusions

Regular consumption of turmeric may prove beneficial in prevention and regulation of diabetic hyperglycaemia if given as an adjunct to already existing diabetic therapies

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