Original Research Article

An Experimental Study to Evaluate the Antihyperglycemic Action of Curcumin in Diabetes Rat Model and Comparison with Glibenclamide

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ABSTRACT

The present study has been conducted with prime aims and objectives to evaluate the antihyperglycemic Curcumin. Second part of study was to compare these effects with those of standard antihyperglycemic drug Glibenclamide. From the observations in the present study, following conclusions were drawn is Curcumin possesses antihyperglycemic actions concordance with the existing literature. However this study needs further strength by doing animal studies and clinical trials before it can be implemented in therapeutics.
Keywords: - Curcumin, Antihyperglycemic, Diabetes mellitus, Glibenclamide.

INTRODUCTION

As many oral antidiabetic agents as well as Insulin cause hypoglycaemia in diabetic patients as side effect, we also have studied hypoglycaemic effects of these drugs in normal nondiabetic rats. Diabetes mellitus is a group of metabolic disorders of multiple aetiologies characterised by chronic hyperglycemia with disturbance of carbohydrate, protein and fat metabolism resulting from defects in insulin secretion or insulin action or both (WHO 1999-Definition of Diabetes mellitus).

It is a chronic illness that requires continuing medical care and ongoing patient self-management education and support to prevent acute complications and to reduce the risk of long-term complications.

India is the Diabetes capital of the world. According to the International Diabetes Federation, 61.3 million people in India had diabetes in 2011. That figure is projected to rise to 101.2 million by 2030. This would mean every fifth diabetic in the world would be an Indian (IDF Diabetes Atlas 2012). The disease is growing fastest in developing countries where there are more people in the lower and middle-income groups. In 2010, the average age-adjusted prevalence of diabetes in India was 8%, higher than that in most European countries. [1] IDF data reveals that India has more diabetes than the United States. In fact, India is ranked second in the world in diabetes prevalence, just behind China.

Both genetic and environmental factors contribute to its pathogenesis, which

involves insufficient insulin secretion, reduced responsiveness to endogenous or exogenous insulin, increased glucose production and/or abnormalities in fat and protein metabolism. The resulting hyperglycemia may lead to both acute symptoms and metabolic abnormalities. DM usually presents with its characteristic symptoms such as thirst, polyuria, blurring of vision, weight loss, and polyphagia. Sometimes its acute complications like ketoacidosis or non-ketotic hyperosmolarity may be the first presentation. These complications in the absence of timely treatment may lead to death. However, the major sources of the morbidity of diabetes are the chronic complications that arise from prolonged hyperglycemia, including retinopathy, neuropathy, nephropathy and cardiovascular diseases.

Diabetes mellitus is a chronic disease which is difficult to cure. Management concentrates on maintaining blood sugar levels as close to normal ("euglycemia") as possible without presenting undue patient dangers. This can usually be with close dietary management, exercise and use of appropriate medications. Insulin only in the case of type 1 diabetes mellitus while oral hypoglycaemic agents such as biguanides and sulfonylureas and/or insulin may be used in the case of type 2 diabetes.

Curcumin is an extract of herb *Curcuma longa* i.e. turmeric, commonly used spice. It has been used as a traditional remedy for many diseases in Ayurveda. Many studies have proved antidiabetic and antihypelipidemic effect of curcumin. In present study, we have evaluated the effect of curcumin on antihyperglycemic.

MATERIALS AND METHODS

The study was conducted in the Department of Pharmacology and Therapeutics, King George's Medical University, Lucknow (Erstwhile Chhatrapati Shahuji Maharaj Medical University). Prior permission was sought from the Institutional Animal Ethics Committee for conducting the study.

Experimental Animals and Rearing Conditions:
18 adult healthy male Wistar rats, of similar physical constitution (in terms of age, body weight), weighing 160-200gm had been used in study. Animals had been obtained from CPCSEA certified animal house [Indian Institute Of Toxicology Research, Lucknow (IITR)]. They were given standard pellet diet and water *ad libitum* and were kept in Institutional animal house under temperature, humidity and light and dark cycle-controlled environment [$25 \pm 2°C$, 70%, 12 hrs' cycle). The animals were housed for two weeks prior to the experiments to acclimatize to new environment. The maintenance of the animals and the experimental procedures were in accordance with the guiding principles of Institutional Animal Ethics committee and the 'Guide for the Care and Use of Laboratory Animals', National Research Council, 1996 (Latest revision in 2011). The guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India were followed.

Drugs and Chemicals
Following drugs and other materials were used in this study. Drugs were given orally in normal saline by gastric gavage with the help of rat feeding cannula and streptozotocin intraperitonealy.

Curcumin: Extract in form of capsules, Dose - 200mg/kg body weight. Eva Green Curcumin, (manufactured by Sir Biotech India.) It was purchased from Eva Lifestyle Private Ltd, Delhi.

Tablet Glibenclamide: Dose - 0.3mg/kg body weight purchased from government authorized medical store.

Injection Streptozotocin: Dose – 30mg/kg body weight in 0.2 ml of citrate buffer (pH 4.5). It was purchased from Merck India Pvt. Ltd.

High Fat Diet: High fat diet was purchased from Dayal Industries Pvt.Ltd., Barabanki Road, Lucknow, Uttar Pradesh; with composed of Crude Fat (Prepared from Rice Bran) (15%); Crude Protein (16%); Acid Insoluble Ash (2.30%); Moisture (8%); Vitamins and Minerals appropriate quantity.

Experimental Design:

The present study had been designed to evaluate the antihyperglycemic action of Curcumin and to compare this with that of standard drug, Glibenclamide. 18 adult male albino wistar rats, randomly divided in 3 groups of 6 rats each, were used. After the acclimatization of 2 weeks, study was started. At day 0 (Week 0), fasting blood samples were taken from each rat from retro-orbital venous plexus under general anaesthesia using Pentobarbitone. Fasting plasma glucose levels were evaluated using the Glucose-Oxidase (GOD) Peroxidase (POD) Method.

Induction of Diabetes Mellitus Type 2

After baseline sampling, 18 rats of diabetic groups i.e. Group 1 to Group 3, were started with exclusive high fat diet and water *ad libitum* for next 4 weeks. At the end of 4 weeks, each rat was given Inj. Streptozotocin in a dose of 30 mg/kg body weight dissolved in 0.2 ml of citrate buffer (pH 4.5) intraperitonealy. One week after Streptozotocin injection i.e. at week 5, fasting plasma glucose level were studied again. Rats with fasting blood sugar level more than 150 mg/dl were considered diabetic. [2] They were continued on high fat diet *ad libitum* for remaining duration.

Animal Grouping

Group 1: Diabetic control Group- No test drugs were given, only normal saline 1ml was given orally daily and were fed with high fat diet for 6 weeks.

Group 2: Curcumin Group- These 6 rats were given Curcumin 200mg/kg body weight dissolved in normal saline, orally daily and high fat diet for 6 weeks.

Group 3: Standard treatment group- These rats were given Glibenclamide 0.3mg/kg body weight, dissolved in normal saline, orally daily and high fat diet for 6 weeks.

At completion of treatment i.e. at 11th weeks, fasting plasma glucose levels were again evaluated.

Statistical Analysis

A one-sample Kolmogorov-Smirnov test was used to investigate whether the variables were normally distributed. The one way analysis of variance (ANOVA) was used to assess the comparability of the groups assigned to the treatment groups. Independent *t*-test/Tuke's pairwise comparison was used to compare the FPG levels between 2 treatment groups. Statistical significance was based on a two-tailed P value < 0.05.

RESULTS

The effects of high fat diet (HFD) and different drugs (Group 1: Control, Group 2: Curcumin, Group 3: Standard-Glibenclamide) on FPG have been summarized in the Table 1. The FPG level in Group 1 was 79.18±6.90 at week 0 which significantly increased to 199.96±19.44 at week 5 (p<0.001), 220.70±20.07 at week 11 (p<0.001). The increase in FPG level from Week 5 to Week 11 was also statistically significant (p<0.001).

The FPG level in group 2 was significantly increased from Week 0 (82.09±9.46) to week 5 (190.63±27.41, p<0.001), week 11 (140.43±16.39, p<0.001) and decreased

from week 5 to week 11 (p<0.001). Almost similar observation was found for Group 3.

Table 1: Effect of Curcumin & Glibenclamide on FPG levels

| Groups | FPG level (Mean±sd) | | | P-value | | |
	Week 0	Week 5	Week 11	Week0 vs week 5	Week0 vs week11	Week5 vs week 11
Group 1	79.18±6.90	199.96±19.44	220.70±20.07	<0.001*	<0.001*	<0.001*
Group 2	82.09±9.46	190.63±27.41	140.43±16.39	<0.001*	<0.001*	<0.001*
Group 3	76.78±9.46	191.22±28.08	105.17±15.44	<0.001*	<0.001*	<0.001*

* Statistically significant

The Table 2 depicts the average percentage change in FPG level from week 0 to follow-ups. There was increase in the level of FPG in all the groups from week 0 to 5 and week 11. However, higher percentage decrease was observed in Group 3 (-45%) as compared to Group 2 (-26.10%) from week 5 to week 11. There was 10.44% increase in the level of FPG in Group 1.

Table 2: Mean Percent Change in FPG level

| Groups | Mean % change in FPG level | | |
	Week 0 to week 5	Week 0 to week 11	Week 5 to week 11
Group 1	152.85	179.27	10.44
Group 3	131.84	71.21	-26.10
Group 5	149.23	37.07	-45.00

DISCUSSION

Diabetes mellitus is a group of metabolic disorders of multiple aetiologies characterised by chronic hyperglycemia with disturbance of carbohydrate, protein and fat metabolism resulting from defects in insulin secretion or insulin action or both. This is associated with an increased risk of complications from vascular diseases.

Insulin resistance and *β cell dysfunction* are two main components of type 2 DM. Type 2 diabetes is characterized by progressive deterioration of normal pancreatic β cells function. In β cells, oxidative glucose metabolism leads to production of reactive oxygen species, which are normally detoxified by catalase and superoxide dismutase enzymes. Persistent hyperglycemia leads to generation of large quantity of reactive oxygen species in these cells. These reactive oxygen species may subsequent damage to cellular components (Robertson et al., 2003). In diabetic, and non- diabetic obese patients, enhanced adipocyte lipolysis raises the FFA [also known as non esterified fatty acid (NEFA)]. In the presence of glucose, fatty acid oxidation in β cells is inhibited and accumulation of long chain acyl co-A occurs (Robertson et al., 2004).

Type 2 DM is frequently associated with hypertriglyceridaemia, one of the major risk factors in development of coronary artery diseases. Hence, Diabetes mellitus is an independent predictor of high risk for CADs. Morbidity due to CADs in diabetics is two to four times higher than nondiabetics. The increased level of triglycerides in Streptozotocin induced diabetic rats observed in present study may be due to lack of insulin, which normally activates the enzyme lipoprotein lipase. Hypertension is frequently associated with type 2 diabetes. Furthermore it is one of the major contributory factors responsible for the long term diabetic complications and associated morbidity/mortality. [3] Maintaining the optimum blood pressure itself reduces the risk of cardiovascular

diseases (heart disease or stroke) by 33% to 50% and the risk of microvascular complications by 33% among the diabetic patients. The present study was conducted to evaluate antihyperglycemic actions of Curcumin. The results of test drugs groups were compared with vehicle control treated group as well as standard drugs treatment group. Glibenclamide was chosen standard treatment drugs for hyperglycemia. Whether Curcumin had any hypoglycemic action in normal rats, was also studied. The galore of studies have proved the efficacy of herb *Curcuma longa,* especially its active constituent Curcumin, in management of DM, dyslipidemia as well as inflammation associated with metabolic syndrome. In our study, we have tried to combine one allopathic drug with a herbal drug and evaluated the effect of curcumin on antihyperglycemic. To best of present knowledge, limited studies have been conducted using such types of combinations. The dose of Curcumin was selected from previous studies. [4]

The diabetes model used was, high fat diet - low dose Streptozotocin induced type 2 DM in adult albino wistar rats. We have chosen the oral route for administering the drugs, as this route is natural, most common and acceptable route for drug administration. In literature, Albino wistar rats have been very commonly used in innumerable studies of diabetes. They were easily available from CPCSEA certified animal house. Hence they were chosen for study.

Antihyperglycemic effects:
Evaluation of the antihyperglycemic effects of test drugs was also started simultaneously (in rats of group 1 to group 3). At beginning i.e. at day 0- weights, FPG levels of these 18 rats were evaluated. Mean values of weights, FPG levels and lipids for every group showed no significant

difference if compared with other groups. The animal model used in present study was 'High fat diet and low dose Streptozotocin induced type 2 DM in wistar rats.' An experimental animal model which aims at mimicking the pathogenesis and clinical features of human type 2 diabetes mellitus should preferably have two traits i.e. insulin resistance and β cell dysfunction. Currently, many studies have reported that the high-fat diet (HFD) feeding rats develop insulin resistance. [5, 6,7] At the same time, low-dose STZ has been known to induce a mild impairment of insulin secretion which is similar to the feature of the later stage of type 2 diabetes. [8, 9] HFD was continued for 6 weeks in diabetic groups (Group 1: Control, Group 2: Curcumin, Group 3: Standard- Glibenclamide) during the curative phase of study. At the end of 6 weeks i.e. at 11th week, FPG.

Curcumin treated groups:
Rats of diabetic group 2, which received Curcumin 200mg/kg body weight for 6 weeks , have shown significant decrease in mean FPG levels {26.10% reduction ,significant ($p<0.001$); table 1 & table 1}. When compared with control, Curcumin has shown significant decrease ($p<0.001$) in mean FPG. These results are consistent with previous studies. [10, 11]
Hypothesis regarding antihyperglycemic action of Curcumin is as follows:
Activation of PPAR-γ
Activation of AMP activated protein kinase (AMPK). [12]
Antioxidant effect. [13]
Results of standard drugs-group (Glibenclamide 0.3mg/kg) have shown more significant decrease in mean FPG (tables 1 & table 2) Standard drugs have been used in such doses that they may produce appropriate result with maximal efficacy. Hence, Curcumin has significant

antihyperglycemic effects. Further studies can be planned using this animal model and including measurement of insulin levels and cytokines levels to strengthen the findings of present study.

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