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ORIGINAL ARTICLE

Outcomes of Cassia Fistula Bark Hexane Extract on Serum Insulin and Glucose Levels in Diabetic Rats

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ABSTRACT

Background: Cassia fistula (amaltas) is commonly found in Pakistan and is frequently used in medicines. Its leaves, bark and pods are conventionally used for the treatment of skin disorders, bacterial infection, constipation, colic, cirrhosis, urinary disorders, dysentery, leprosy, rheumatic conditions, jaundice, syphilis, heart diseases and diabetes mellitus (DM).
Aim: To determine and compare the outcomes of cassia fistula bark (hexane extract) on serum insulin and blood glucose measures in *high-fat diet-fed* (HFD) and *Streptozotocin-induced type 2 diabetic* albino male rats."
Study design: Randomized control trial.
Duration and setting: Services Institute of Medical Sciences (SIMS), Lahore from August 2016 to January 2017.
Methods: The research was carried out on ninety male albino rats. Rats were induced with diabetes by giving high fat diet for 2 weeks and on fourteenth day, single injection of low dose streptozotocin (25mg/kg). The rats were categorized randomly into two groups, randomly, of 45 animals each. Group 1 was diabetic control (n=45) and group 2 was diabetic experimental and was given hexane extract of *Cassia fistula* bark (0.45g/kg) in 1ml of 0.3 % Carboximethyl cellulose solution by using intra gastric tube for once in a day for a month. On 31st day, 4-5 ml intra cardiac blood sample was collected. Biochemical parameters serum glucose and insulin were evaluated.
Results: The diabetic rats treated with hexane extract of cassia fistula bark, exhibited significant (p=0.05) decrease in serum insulin and glucose levels.
Conclusion: Hexane extract of cassia fistula bark possesses potent hypoglycemic effects.
Keywords: Cassia fistula, Hypoglycemic effects, diabetic rat

INTRODUCTION

Diabetes mellitus is a disorder of metabolic nature and is manifested as chronic hyperglycemia with disturbance of carbohydrate, fat and protein metabolisms, which in turn results from defective insulin action, secretion, or both. This disorder is broadly categorized into four classes such as type-I diabetes mellitus occurs due to immune mediated or idiopathic beta cell destruction ending up in absolute insulin deficiency, type II diabetes mellitus may results due to insulin resistance along with relative insulin deficiency and insulin secretary problems, other specific types of diabetes, for instance, genetically abnormal β cell function, abnormal insulin action, disorders of exocrine pancreas (such as cystic fibrosis) and gestational diabetes mellitus[1]. This genetically and clinically heterogeneous group of disorders exhibits common features of hyperglycemia, hyperlipidemia and oxidative stress injury[2,3].

Globally different epidemiological researches and clinical studies potentially support the idea that hyperglycemia is the major factor to cause coronary artery disease, cerebrovascular morbidities, renal complications, neurological diseases, premature deaths and abnormalities in lipid metabolism in diabetics[4]. Patients having type 2 diabetes mellitus have shown increased incidence of long term complications such as atherosclerotic cardiovascular, peripheral arterial and cerebrovascular diseases[1,5]. Hyperglycemia and deranged lipid profile particularly related with type II diabetes mellitus is typically manifested by raised triglycerides, decreased density lipoproteins and decreased high density lipoprotein have proved to be the risk factors for macrovascular and microvascular outcomes of disease[6].

Medicinal plants have always been stayed in mainstay for treating and preventing a variety of diseases throughout the world. Conventionally various medicinal plants have played important role worldwide as remedy of diabetes mellitus. These remedies may slow down the development of diabetic complications and act as a source for antioxidants to prevent or delay various stages of this disease[7]. Herbs may act as a source of increasing insulin secretion as well as help to enhance glucose entry into adipose cells and skeletal muscles, preventing glucose absorption in intestines and inhibit glucose production by hepatocytes in liver[8]. One of the traditional medicinal plants for diabetes mellitus, *Amaltas (Cassia fistula)*, has gained worldwide prominence in recent past, because it has a wide range of medicinal properties. *Cassia fistula* has been broadly recommended in conventional medicine in India. Almost all parts of *Cassia fistula* are accounted for anti-inflammatory, antitussive, antimicrobial, anticancerous, hepatoprotective, antioxidant, and antibacterial activity[9].

The aims of this research were to evaluate and compare the results of hexane extracts of bark of cassia fistula on blood glucose and insulin levels in male diabetic albino rats.

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MATERIAL AND METHODS

Ninety male healthy rats were attained from NIH. Each cage had 30 animals, which were kept there for 7 days before the commencement of evaluation. Housing conditions were kept at a temperature of 26±2 0C and 12 hour light/dark cycle[10]. The animals were offered commercially prepared standard pellet diet ad libitum and they had clean-bottled tap water to drink. The bottles had a valve like mechanism at their one end through which animals could suckle water.

Development of diabetic rat model: In present study, 90 rats were given high fat diet ad libitum, initially for 2 weeks. On day 14th, HFD rats, after 12 hours fasting, were given a single dose of streptozotocin (25mg/kg). With this dose of streptozotocin, all β cells were not to be destroyed so it induced type II diabetes mellitis[11]. Normal fasting plasma glucose levels in rats ranges from 80-110mg/dl[12]. Rats having blood glucose ≥ 200mg/dl and marked as diabetic were included in the study13. Confirmation of diabetes was established by estimating fasting plasma glucose by a glucometer using blood samples from the tail vein after 96 hours (4

days) of streptozotocin administration[14].Hyperglycemia was established by raised blood glucose, measured after 72 hours. Rats with blood glucose>200 mg/dL were considered diabetic and included in experiment. Group II HFD+STZ induced diabetic rats were put on *Cassia fistula* (at a dose of 0.45g/kg body weight) in 1ml of 0.3% Carboximethyl cellulose per day for a total of 30 days[14].

Plant material and extraction: Hexane extract of *Cassia* bark was prepared and standardized while using facilities available at *Applied Chemistry Research Centre, PCSIR labs, Lahore*. Freshly obtained bark of the said plant was cut, dried in shade and roughly powdered with the help of electric grinder. The extract was then dried under decreased pressure created by rotatory vacuum evaporator. After evaporation with rotatory evaporator, a brownish concentrate was collected. The actual percentage yield was 7% w/w and extract was stored at 4°C before using it[14]. The crude extract was then dissolved in sterilized distilled water to achieve a dilution of the desired concentration[10,16].

Grouping: Ninety rats were haphazardly splitted into 2 groups

Group I: (Control, n= 45): HFD-STZ diabetic rats in this group had normal diet and water available ad libitum and they also received 1ml of 0.3% Carboximethyl cellulose solution every day with intragastric tube for a period of 30 days[14].

Group II: (Experimental, n=45): HFD+STZ diabetic rats were given *Cassia fistula* extract (0.45g/kg body weight) in 1ml of 0.3% Carboximethyl cellulose solution every day with oral tube for a period of 30 days[14].

After 31st day of the study, 4-5ml of blood sample was collected intracardiac for determination of plasma glucose levels by glucose oxidase method to observe the in-vivo effect of the plant extract.**:** Initial blood samples were drawn aseptically 96 hours after administration of streptozotocin. For terminal sampling on 31st day, overnight fasted rats were anaesthetized by ether inhalation. 5 ml blood was drawn through cardiac puncture from each rat and immediately transferred to the gel vials with identification numbers. Then, blood sample was subjected to centrifugation at a speed of 5000 rpm for approximately 5 minutes and very carefully serum was separated for determination of blood glucose level.

Analysis of statistical data: The data was scanned by using PASW 18 (known as SPSS). Expected values of quantitative variable like serum glucose and insulin were calculated. A student 't'test was applied to determine statistical significance of difference between different variables amongst the two groups. Significant p value will be ≤ 0.05 and highly significant if the p value will be ≤0.01.

RESULTS

This randomized controlled research was conducted on 90 rats. The effects of *Cassia fistula* bark on serum insulin and glucose were strategically calculated and compared among the diabetic male albino rats. Serum glucose and insulin levels of two groups are depicted in Table 1. Student 't' test showed that the mean±SD serum glucose and insulin of group ll (209.46±12.37 mg/dl, 67.44±6.89 µU/ml) were significantly lower as compared to that of control group values (214.20±6.06mg/dl, 70.51±3.62µU/ml) (p=0.01,0.02).

Table 1: Comparison of serum glucose and insulin between diabetic control and diabetic experimental groups

Parameters	Group I	Group II	p. value
Serum glucose(mg/dl)	214.20±6.06	209.46±12.37	0.02*
Serum insulin(µU/ml)	70.51±3.62	67.44±6.89	0.01*

Values are mentioned as *Mean ± SD*

*p value ≤0.05 is considered significant

DISCUSSION

Study under consideration has been undertaken with the aim to manifest the hypoglycemic effect of cassia fistula bark in rats who had taken high-fat diet thoroughly to produce the resistance for insulin at tissue level and increase the blood glucose level but markedly increase blood glucose levels were achieved by giving single intraperitoneal streptozotocin injection. This is first report which highlights the antidiabetic effects of cassia fistula bark in this unique type 2 diabetic rat model that would firmly reveal the logical history and metabolic effects of human diabetes mellitus type 2.

The research was managed on ninety rats. Initially they were provided with HFD for a total of 14 days, that evolved resistance against insulin in rats tissue due to which rats were gained weight (obesity), slight increased glucose levels that resulted in hyperinsulinaemia. Rats which were already mildly hyperglycemic due to resistance of insulin became more vulnerable to the diabetes causing effect of low dose STZ (25mg/kg). Specifically, beta cells of pancreas under the stress of compensatory hyperinsulinemia could be easily impaired by lower doses of STZ.

Type 2 diabetic rats manifested increase in serum glucose and serum insulin levels due to increased deposition of fat on tissues. These results are analogous to those of Veerapur et al (2012), who also utilized this type 2 diabetic rat model that resulted in increased serum glucose and serum insulin levels[11]. Current study has successfully established that treatment with hexane extract of cassia fistula bark (0.45g/kg) for 30 days turned out remarkable (p=0.01) decrease in glucose and serum insulin levels (p=0.02).

These citation are comparable with the findings of Nirmala et al (2008) who also employed extract of cassia fistula bark at doses 0.15, 0.30 and 0.45g/kg body weight in diabetic rats, which then suppressed the elevated levels of serum glucose and insulin in those diabetic rats. The extract amount of 0.45 g/kg was in accordance with glibenclamide, the reference drug[14].

A comparable study also was designed by Ali et al (2012), to evaluate "anti hyperglycemic effects of ethanolic extract of cassia fistula bark" in rats. Hypoglycemic effect was checked with the help of oral glucose tolerance test (OGTT) in normal and treated diabetic rats and metformin (150mg/kg, p.o.) was used as recommended drug for correlation. The extract prevented hyperglycemia chances appreciably (P<0.05), in alloxan induced diabetic and normo-hyperglycaemic rats orally (at 250 and 500 mg/kg body weight respectively). The glucose tolerance test results showed notably (p<0.05) result.

Garba et al, also evaluated hypoglycemic potential of cassia fistula bark by treating normal and diabetic rats. The out turn of the "methanolic cassia root extract on weight and selected biochemical parameters were assessed. Intraperitoneal injection of of alloxan with dose of 150mg/kg body weight was given to induce diabetes. When diabetes was confirmed, extracts at three different dose levels and metformin (standard drug) were given daily to diabetic rats for a duration of 14 days. The experiment continued for 21 days and blood glucose levels were done at day 0, 7, 14 and 21. Diabetic rats treated with the extract showed effective results in blood glucose levels. In this study, ideal hypoglycemic activity was noticed in the rat group treated with 400mg/kg b.w of *Cassia fistula* root extract and this was not different remarkably from that of metformin-treated rats[16]. The research under consideration shows that the hexane extract of bark has anti-hyperglycemic potential. This outcome scientifically proves its utilization in regular medicine for treatment of diabetes mellitus[17].

To summarize, the outcome of our research strongly suggest that Cassia fistula bark can effectively be used as a treatment of diabetes

CONCLUSION

Current study concludes that Hexane extract of cassia fistula bark possesses hypoglycemic effects.
Conflict of interest: Nil

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