Research Article

Effect of some Indian herbs on dyslipidemia in streptozotocin induced diabetic Rats

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ABSTRACT

Background: *Diabetes is one of the commonest and serious metabolic disorders. Much of the morbidity and mortality associated with diabetes is primarily attributed to sequelae requelac of microvascular and macrovascular changes, in which diabetic dyslipidemia is one of the many modifiable risk factors for coronary artery disease, stroke and peripheral vascular disease.*

Objectives: *The main aim of this study was to evaluate the hypoglycemic and antidyslipidemic effect of selected Indian plants in streptozotocin induced diabetic rats.*

Material and Methods: *Azadirachta indica and Ocimm sanctum leaves, Allium sativum bulbs and Momordica charantia fruits were collected, identified taxonomically and extract was obtained. Male Albino rats was used and divided into 8 groups, each consisting of 6 animals, one group act as a control. Diabetes in rats was induced with streptozotocin. Blood samples were collected and biochemical analysis was done for blood sugar, lipid peroxide and lipid profile. The diabetic group without drug treatment was compared with the control, and diabetic plus drug-treated groups were compared with the diabetic group without drug treatment. Data were analyzed using Student 't' test.*

Results: *Our results revealed that administration of streptozotocin in rats caused increase in the levels of glucose, lipid peroxides, cholesterol and triglycerides with lessening of the HDL-cholesterol. Treatment with aqueous extracts of Momordica charantia, Allium sativum, Azadirachta indica and Ocimum sanctum not only reduced the level of blood glucose but also caused lowering of total cholesterol and triglycerides following an increase in the level of HDL-cholesterol.*

Conclusion: *We concluded that the herbal plants tested possess both hypoglycemic and antidyslipidemic activities and their use as a therapeutic tool in diabetes related complications encourage further investigation in this field.*

Key words: *Diabetes, dyslipidemia, Indian herbs, lipid peroxidation*

Introduction

Diabetes is rapidly emerging as a global health problem which could reach pandemic levels by 2030. The number of people with diabetes worldwide is projected to increase from 171 million in 2000 to 366 million by 2030. [1] The perceptible increase will be most in developing countries. Patients with diabetes are at risk of increased morbidity and mortality from both macro and microvascular complications. Hyperglycemia and dyslipidemia which generally coexist in diabetic patients are significant and independent risk factors for the vascular complications in them. [2] About 18 million people die every year from cardiovascular diseases, for which diabetes and hypertension are major predisposing factors. [1] In particular, the interaction of

hyperglycemia and dyslipidemia increases the risk of diabetic complications synergistically by altering vascular cellular metabolism, vascular matrix molecules and circulating lipoproteins. For instance hyperglycemia increases diacylglycerol levels and activates protein kinase C activity in the aorta of streptozotocin induced diabetic rats.

Since dyslipidemia in patients with metabolic syndrome including diabetes may independently contributes to higher risk of atherosclerosis and subsequently to cardiovascular diseases, therefore diabetes related changes in plasma lipid concentrations are amenable to intervention. [3]

The oxidative stress and resultant tissue damage are hallmark of chronic diseases and cell death and diabetes is no exception. Oxidative stress combined with mitochondrial dysfunction leads to the activation of inflammatory signaling pathways which may damage insulin-producing cells and further aggravate the complications of diabetes. [4]

Since time immemorial, individuals with diabetes have been treated orally with folk medicine of a variety of plant extracts. Recently, there has been increasing interest in the use of medicinal plants. The plant kingdom has become a target for multinational drug companies and research institutes for the discovery of new biologically active compounds and potential drugs. The World Health Organization has recommended, especially in developing countries, the initiation of programmes designed to use medicinal plants more effectively in the traditional healthcare system. The resolution of the 31st World Health Organization Assembly requested a complete inventory, and a thorough evaluation of the efficacy, safety and standardization of medicinal plants for the treatment of diabetes. [5]

Ethnobotanical information indicates that more than 1200 plants have been used as traditional remedies for the treatment of diabetes. The antihyperglycaemic activity of a large number of these plants has been evaluated and confirmed in different animal models. In addition to correction of blood glucose levels, several hypoglycaemic plants are potential in ameliorating lipid metabolism abnormalities of diabetes mellitus. [6] Thus, the study of hypoglycaemic and antidyslipidemic activities of a plant may give new pharmacological approach in the treatment of diabetes mellitus.

Moreover, recently diet and spice therapies have become the major approaches being proposed for the treatment and control of diabetes and a considerable amount of work has been carried out in this regard with *Momordica charantia, Allium sativum, Azadirachta indica and Ocimum sanctum.* [7, 8] All of these plants possess potent hypoglycemic activity; however in this connection we have made an attempt to evaluate the antihyperglycemic and antidyslipidemic actions of these natural products on blood glucose and serum lipid profiles in streptozotocin induced diabetic rats.

Material and Methods

Plant material

Azadirachta indica and *Ocimm sanctum* leaves were collected from CSM Medical University Campus, Lucknow whereas *Allium sativum*

bulbs and *Momordica charantia* fruits were purchased from a local market in Lucknow. All the plants were identified taxonomically by the Department of Botany, Shia P.G. College, Lucknow.

Preparation of crude extract

A sativum

Fresh garlic bulbs were cut into small pieces, and 250 ml of triple-distilled water (TDW) per 100 g of garlic was added and crushed in a mixing machine. The resultant slurry was squeezed and filtered through a fine cloth. The filtrate (extract) was quickly frozen. [9] A portion of the extract was freeze-dried, which yielded 28.7 g (wt/wt) of solid mass.

A indica

Air-dried plant leaves (100 g) were boiled in 200 ml of TDW for 10 minutes. After cooling to room temperature, the supernatant was filtered to obtain the decoction ready for animal treatment. [10] This extract contained 10.0 g (wt/wt) of solid mass.

M charantia

Fresh fruits (250 g) were taken and the seeds were removed. The fleshy parts were cut into small pieces and macerated with 250 ml TDW using a mortar and pestle. This suspension was squeezed through a muslin cloth, and the liquid was centrifuged at 5000 rpm for 30 minutes in the cold. The supernatant used for study contained 25 g (wt/wt) of solid material. [11]

O sanctum

Air-dried leaves powder (100 g) was boiled in 200 ml TDW for 10 minutes. After cooling to room temperature, the supernatant was filtered to obtain the decoction ready for animal treatment. [12] This contained 10 g (wt/wt) of solid mass.

Glibenclamide

This compound was purchased from Sigma Chemicals (St Louis, Mo, USA). The compound was sonicated at 20 kHz for 1 x 3 minutes in TDW, and 0.5 mg/ml suspension was prepared. This was mixed thoroughly just before use.

Insulin

Insulin was purchased from Torrent Pharmaceutical Ltd, Mumbai, India, and administered as such intraperitoneally once daily for 30 days.

Chemical Used

Streptozotocin (STZ) was purchased from Sigma Chemical co. (St Louis Mo, USA). Other chemicals used were of high purity and analytical grade.

Animals and treatments

Male albino Sprague-Dawley rats weighing 180 to 200 g were kept at 20^0C in a room of the Animal House, Era,s Lucknow Medical College & Hospital, Lucknow. The animals were housed in polypropylene cages and kept in uniform hygienic condition. They were provided standard pellet diet (Ashirwad, Industries, Ltd, Chandigarh, India) and water *ad libitum*. Prior permission for animal use and approval of the protocol were obtained from the Institutional Animal Ethics Committee. Rats were divided into the following 8 groups, each consisting of 6 animals. The test herbal preparations as well as glibenclamide and insulin were administered once a day for 30 days to their effective doses as reported earlier and described below.

- Group 1 Control fed with isotonic sodium chloride solution
- Group 2 Streptozotocin-induced diabetic fed with isotonic sodium chloride solution
- Group 3 Diabetic treated with *A. sativum* (10 ml/kg b.wt.) [9]
- Group 4 Diabetic treated with *A. indica* (10 ml/kg b.wt.) [10]
- Group 5 Diabetic treated with *M. charantia* (10 mg/kg b.wt.) [11]
- Group 6 Diabetic treated with *O. sanctum* (10 mg/kg b.wt.) [12]
- Group 7 Diabetic treated with insulin (5 units/kg b.wt.) [13]
- Group 8 Diabetic treated with glibenclamide (5 mg/kg b.wt.) [13]

Diabetes in rats was induced with a single injection of streptozotocin (65 mg/kg body weight) by intraperitoneal route. [8] Diabetes was confirmed by the determination of fasting blood glucose concentration with the help of a glucometer on the third day after administration of streptozotocin. The animals with blood glucose levels from 180 to 200 mg/dl were segregated and kept into cages marked with groups 2 to 8. The body weights of all the rats were determined on the first and 30th days of the experiment. The drug preparations were fed orally by gastric intubation to rats of respective groups (groups 3-8) once daily for 30 days. Control animals (groups 1-2) received the same amount of isotonic sodium chloride solution. The animals were treated with insulin & glibenclamide (groups 7-8) since these are the recognized drugs for the treatment of diabetes mellitus & have a well documented effect on lipid profile levels.

Blood collection and biochemical analysis

At the end of the experiment (30 days), rats were fasted overnight and anesthetized with sodium pentothal (intraperitoneally); and 4 ml of blood was withdrawn through the retro-orbital plexus using a glass capillary. Blood was collected in EDTA-coated vials and kept in ice- water for 30 min. This was centrifuged for 10-minutes at 1500×g. The plasma was aspirated out and used for further estimations.

Estimation of lipid peroxides by the method of ohkawa et al

The tubes containing 0.5ml of plasma samples were subsequently mixed with 0.5 ml of glacial acetic acid, 0.5 ml of 8% sodium dodecyl sulfate and 0.8% thiobarbituric acid (TBA) and stirred well after addition of each reactant. The reaction mixture was then kept in a boiling water bath for 1 hour. After cooling to room temperature, 3.0 ml of n-butanol was mixed and vortex vigoursouly. The reaction mixture was transferred into centrifuge tubes and spinned at 11000×g for 15 minutes. The absorbance of the clear butanol fraction obtained after centrifugation was measured at 532 nm in a spectrophotometer (Spectronic 21; Milton Roy, Ivyland, Pa). An appropriate standard solution of malondialdehyde (2.5 nmol) was also run simultaneously.

Determination of blood glucose level by the method of Trinder

To determine glucose level, 20 µl of plasma was added to 0.2 ml TDW and 3 ml of color reagent. The reaction mixture was incubated at 37^0C for 15 minutes simultaneous with tubes containing the reagent blank and standard glucose 10 µl (10 µg). The absorbance of color developed was

measured by the spectrophotometer at 505 nm against the reagent blank. The composition of the coloring reagent was a mixture of 4-aminoantipyrine 0.5 mmol, p-hydroxybenzene sulfonate 20 mmol, glucose oxidase 15 000 U/L, and peroxidase 10000 U/L brought to a final volume of 1L with 100mM phosphate buffer at pH 7.00.

Measurement of Lipid Profile

Total Cholesterol (TC) and triglycerides (TG) were measured in plasma by using colorimetric method with commercially available kits (Transasia Biomedicals Ltd., Germany). A portion of plasma was precipitated with phosphotungstic acid/ $MgCl_2$. After centrifugation clear supernatant containing soluble high density lipoprotein (HDL) was estimated for HDL-cholesterol. Very low and low density lipoprotein (VLDL and LDL) cholesterol was calculated according to Frieldwald formula.

- VLDL-cholesterol = Plasma TG/5
- LDL-cholesterol = Plasma TC- (HDL chol + VLDL chol)
- All the values are in mg/dl plasma.

Data were analyzed using Student 't' test. The diabetic group without drug treatment was compared with the control, and diabetic plus drug-treated groups were compared with the diabetic group without drug treatment. The values were tested for significance and P value <0.05 was considered significant.

Results

The effect of herbal hypoglycemic agents on body weight, fasting blood glucose, plasma lipid peroxide levels & plasma lipid profile in normal and diabetic rats was studied. A significant decrease in fasting blood glucose and improved body weight was observed in groups 3-8, however the maximum effect was in group 3 (57%) as shown in table- 1. Elevated plasma lipid peroxide levels, by about 3-fold (+194%) were found in group 2 as compared to group 1. After drug treatment maximum reduction in plasma lipid peroxide levels was observed in group 6. Administration of the herbal extracts for one month significantly ($p<0.001$, $p<0.01$) improved hypertriglyceridemia and hypercholesterolemia in groups 3-6 as also was the case with group 7(insulin treated) and group 8 (glibenclamide treated) animals.

The results of the present study showed that group 2 (diabetic rats) had higher concentration of serum TC (+44.32%) and TG (+54.71 %) than group 1 (controls). The levels of these lipids were significantly reduced after treatment with above drugs but with varying extent. The reduction was more pronounced in groups 3&5 (23.21 and 22.46% respectively) for TC and group 4 (27.05%) for TG levels. Administration of STZ also caused marked increase in VLDL-cholesterol (+54.82%) and LDL-cholesterol (+204.4%) in rats. After treatment with herbal drugs maximum reduction in VLDL-cholesterol was found in group 4 (27.05%) & LDL-cholesterol in groups 3 & 5 (47.15%, 45.12 % respectively). The levels of HDL-cholesterol were lowered in group 2(diabetic rats) as compared to group 1(controls). Treatment with the herbal hypoglycemic agents recovered the levels of HDL-cholesterol to some extent which was more pronounced in group 3 (52.19%).

Table 1: The effect of herbal hypoglycemic agent on body weight, blood glucose and plasma lipid peroxide in normal and diabetic rats

Para-meters	*Group 1 (control)*	*Group 2 (diabetic)*	*Group 3 (diabetic + A sativum)*	*Group 4 (diabetic + A indica)*	*Group 5 (diabetic + M charantia*	*Group 6 (diabetic + O sanctum)*	*Group 7 (diabetic + insulin)*	*Group 8 (diabetic +glibencla-mide*
% Change in body weight (g)	+31.0 ± 1.12	-16.5 ± 2.2***	+5.3± 1.4**	+4.5 ± 1.05**	+6.4 ± 3.2**	+6.8 ± 0.84**	+12.3 ± 2.4*	+10.2± 1.35*
Glucose (mg/dl)	82.06 ± 4.32	199.48 ± 9.78*** (+143.09)	85.39 ± 17.12*** (-57.19)	94.45 ± 2.48*** (-52.65)	105.25 ± 6.58*** (-47.23)	92.15± 24.00*** (-53.80)	97.42 ± 4.03*** (-51.16)	105.92 ± 8.25*** (-46.90)
Plasma lipid peroxide (nmol/mL)	2.98 ± 0.373	8.79 ± 0.564*** (+194.96)	3.60 ± 0.759*** (-59.04)	3.36 ± 0.037*** (-61.77)	3.39 ± 0.126*** (-61.43)	2.97 ± 0.090*** (-66.21)	3.19 ± 0.071*** (-63.7)	3.81 ± 0.239*** (-56.65)

Diabetic group without treatment (group 2) was compared with control (group 1) and diabetic plus drug treated (group 3-8). The change in body weight was the percentage change measured just before euthanizing the rat and was compared with the weight taken before the start of the experiment. Groups follow those presented in Material and Methods Values in the parentheses are percent change *** P <0.001. ** P <0.01. * P <0 .05

Table 2: The effect of herbal hypoglycemic agent on plasma lipid profile in normal and diabetic rats

Parameters	*Group 1 (control)*	*Group 2 (diabetic)*	*Group 3 (diabetic + A sativum)*	*Group 4 (diabetic + A indica)*	*Group 5 (diabetic + M charantia*	*Group 6 (diabetic + .O sanctum)*	*Group 7 (diabetic + insulin)*	*Group 8 (diabetic + glibenclamide*
Total cholesterol	82.80 ±3.27	119.5 ±5.58*** (+44.32)	91.76 ±3.71*** (-23.21)	100.9 ±4.44*** (-15.56)	92.65 ±3.32*** (-22.46)	97.6 ±6.28*** (-18.32)	96.72 ±4.11*** (-19.06)	99.72 ±3.75*** (-16.5)
Triglyceride	71.55 ±3.49	110.7 ±6.51*** (+54.71)	90.41 ±17.26* (-18.32)	80.75 ±20.32** (-27.05)	90.03 ±11.18** (-18.67)	100.96 ±9.58* (-8.79)	80.38 ±7.11*** (-27.38)	90.21 ±3.37** (-18.50)
HDL-C	45.18 ±2.51	26.40 ±3.42*** (-41.56)	40.18 ±5.44*** (+52.19)	33.14 ±4.28** (+25.53)	35.71 ±5.22* (+35.26)	30.97 ±4.42* (+17.31)	43.14 ±3.57*** (+63.40)	43.43 ±5.58*** (+64.50)
VLDL-C	14.30 ±0.70	22.14 ±1.42*** (+54.82)	18.08 ±3.78* (-18.33)	16.15 ±4.45** (-27.05)	18.00 ±2.45* (-18.69)	20.13 ±2.10* (-9.07)	16.76 ±1.55*** (-24.29)	18.42 ±0.675*** (-16.80)
LDL-C	23.31 ±4.10	70.96 ±7.07*** (+204.4)	37.5 ±4.82*** (-47.15)	51.61 ±7.16*** (-27.26)	38.94 ±9.20*** (-45.12)	46.50 ±10.25** (-34.47)	36.82 ±7.6*** (-48.11)	37.87 ±6.92*** (-46.63)

Values are means ± SEM for 6 rats. Diabetic group without treatment (group 2) was compared with control (group 1) and diabetic plus drug treated (group 3-8). Groups follow those presented in Methods and Materials. Values in the parentheses are percent change with various treatments *** P <.001. **P <0.01. *P <0 .05

Discussion

There are reports that hyperglycemia, dyslipidemia with increased level of circulatory free fatty acids, equally contribute to initiation as well as progression of diabetes related complications; retinopathy, neuropathy, renal and cardiovascular

diseases in patients. [14] Disorders of lipid metabolism following induction of oxidative stress are known to aggravate the Coronary artery disease.

The present study was an effort to investigate the effect of some herbal plants on diabetic dyslipidemia induced by STZ in rats. The study revealed a significant increase ($P < 0.001$) in fasting blood glucose and decrease ($P < 0.001$) in body weight of diabetic rats without treatment with test samples (group 2). The decrease in body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins. Treatment with *A sativum, A indica, M charantia, O sanctum*, insulin, and glibenclamide in diabetic rats caused a significant decrease in blood glucose levels and improved body weight but with varying extent. This could be due to a better control of the hyperglycemic state in diabetic rats. The highest effect observed was with *A sativum* (57%), which was more efficient to that of insulin and glibenclamide. Simultaneously, recovery in body weight of animals was also found after treatment (Table 1). Parenteral administration of insulin is well known to cause hypoglycemia in normal as well as streptozotocin induced diabetic rats. [15] Glibenclamide, one of the most widely used oral hypoglycemic agents in the treatment of diabetes mellitus, exerted its beneficial effects on extracellular site by opening Ca^{2+} channels to stimulate insulin secretion and also duodenal insulin-releasing agent. However, the hypoglycemic action of these herbal preparations may be due to their extra pancreatic sites of action, that is, by direct metabolic effect on tissues, particularly liver. The constituents of *A. sativum* [16] and *O. sanctum* [17] have been reported to exert stimulatory effects on physiological pathways in insulin secretion, which may explain the antidiabetic action. The results of our study also demonstrated elevated plasma lipid peroxide levels by about 3-fold (+194%) in the diabetic group as compared to controls. These results are in concordance with previous studies in humans showing elevated plasma lipid peroxide levels in diabetic subjects. [18] Increase in lipid peroxide levels in plasma is said to be one of the most important contributing factors for the development of diabetes-related complications. [19] However, in the present study, treatment with above mentioned herbal drugs caused significant reduction in plasma lipid peroxide levels in diabetic rats, and this effect was seen highest with *O. sanctum* (66%).

The most common lipid abnormalities in diabetes are elevated levels of circulating FFA and hypertriglyceridemia predominantly in VLDL. The abnormally high concentration of serum lipids in diabetes mellitus is mainly due to an increase in the mobilization of free fatty acids from the peripheral fat depots. Since insulin, apart from regulation of carbohydrate metabolism acts as an antilipolytic agent and regulates the catabolism or storage of fat in body therefore decreased bioavailability of this hormone (as in diabetic) inforces the action of lipolytic enzymes on stored fat in adipose tissue. The breakdown of TG thus markedly elevate the levels of circulatory FFAs, some of this may

incorporate back as VLDL-TG. Hypertriglyceridemia is also associated in metabolic consequences of hypercoagulability, hyperinsulinemia, insulin resistance and glucose intolerance. [20] Administration of the herbal extracts for one month significantly ($p<0.001$, $p<0.01$) improved hypertriglyceridemia and hypercholesterolemia, as was the case with insulin and glibenclamide which were also very effective in improving all these parameters.

The results of the present study showed that streptozotocin induced diabetic rats had higher concentration of serum TC (+44.32%) and TG (+54.71 %) than respective non-diabetic rats, however, levels of these lipids were significantly reduced after treatment with above drugs but with varying extent. The reduction was more pronounced with *A. sativum* and *M. charantia* (23.21 and 22.46% respectively) for TC and *A. indica* (27.05%) for TG levels. Administration of STZ also caused marked increase in VLDL-cholesterol (+54.82%) and LDL-cholesterol (+204.4%) in rats. Treatment with *A. indica* acted upon VLDL and reduced the cholesterol by 27.05% whereas *A. sativum* and *M. charantia* reduced the LDL-cholesterol by 47.15 and 45.12% respectively, in diabetic animals.

The results of the present study also showed that, diabetic rats had lower HDL-cholesterol concentration, than respective non-diabetic rats. Treatment with herbal hypoglycemic agents recovered the levels of HDL-cholesterol to some extent. The maximum effect of increment was observed with the treatment of *A. sativum* (52.19%). The observed hypolipidemic effect may be due to decreased cholesterologenesis and fatty acid synthesis. The hypolipidemic effect of insulin and glibenclamide was more marked; because these drugs are very effective for regression of hyperglycemia, the primary cause to induce dyslipidemia in diabetes. [7] Significant lowering of TC, total lipids and TG and rise in HDL-cholesterol is a very desirable biochemical state for prevention of atherosclerosis and ischaemic conditions [21].

On the basis of results of our study it may be concluded that these herbal hypoglycemic agents are not only useful in reducing the blood glucose level but they are also found to be highly effective in managing the complications associated with diabetes mellitus such as hyperlipidemia and prevent the defect in lipid metabolism. Therefore above mentioned herbal plants show therapeutic role as a protective agent against the development and progression of atherosclerosis and possible related cardiovascular complications in diabetes mellitus.

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