ORIGINAL ARTICLE

Duration effect of Acacia nilotica leaves extract and glibenclamide as hypolipidaemic and hypoglycaemic activity in alloxan induced diabetic rats

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Abstract

Objectives: To compare the duration and effects of aqueous methanol Acacia-nilotica leaves extract and glibenclamide as hypoglycaemic and hypolipidaemic activity in diabetic rats.

Methods: The experimental study was conducted at Shifa International Hospital in collaboration with National Institute of Health, Islamabad, from September 2010 to August 2011.Male Sprague Dawley albino rats were taken and divided into 8 equal groups. Groups I and II were the normal and diabetic control rats. Diabetes mellitus was induced in group II to VIII by administering 110 mg/kg body weight alloxanand at day 4, fasting blood glucose level of >200 mg/dl confirmed diabetes. Acacia-nilotica leaves extract was given to group III, IV and V and glibenclamide to group VI to VIII for a period of 1-3 weeks. Blood samples were analysed for lipid profile using enzymatic calorimetric method and serum insulin by enzyme-linked immunosorbent assay on days 0, 7, 14, and 21.

Results: There were 64 rats in the study, with 8(12.5%) in each group. Statistically significant decreases in fasting blood glucose, total cholesterol, triglyceride, phospholipids, low density lipoprotein, very low density lipoprotein and an increase in high density lipoprotein and serum insulin levels were observed in diabetic rats compared to diabetic controls after 2 weeks of treatment with plant extract and glibenclamide ($p<0.05$ each).When plant extract and drug treated diabetic rats were compared, a significant difference in the levels of blood glucose, insulin, total cholesterol and triglyceride levels were noted after 2 and 3 weeks of treatment.

Conclusion: Acacia-nilotica leaves extract resulted in hypoglycaemic and hypolipidaemic effect in alloxan-induced diabetic rats similar to glibenclamide.

Keywords: Acacia-nilotica, Alloxan, Glibenclamide, Hypoglycaemia, Hypolipidaemic. (JPMA 65: 1266; 2015)

Introduction

Diabetes mellitus (DM) is the syndrome of disturbed energy homeostasis caused by abnormal metabolisms of carbohydrates, proteins and fats. It is the most common endocrine-metabolic disorder of childhood and adolescence with important consequences on physical and emotional development.[1]

Globally, in 2010, about285 million people had diabetes, and, according to International Diabetes Federation, this number increased to 381 million. In 2013[2] while by 2030, the number of diabetics is estimated to almost double. Type 2 diabetes mellitus (T2DM) is most common (90% of the diabetics) and is seen more commonly in the more developed countries. The greatest increase in prevalence is, however, expected to occur in Asia and Africa. The increase in incidence in developing countries seems to be due to a change in lifestyle and a trend of urbanisation, especially of Western-style diet suggesting an environmental effect, but there is little understanding of the mechanism(s) at present.[3]

According to World Health Organisation (WHO), 12.9 million people were diagnosed as diabetics in Pakistan in 2011. It is estimated that Pakistan is 7th largest country of world in terms of diabetes population and will be on 4th number after China, India, and USA by 2030.[4]

The oxidative stress (OS) plays an important role in the pathogenesis of micro- and macro-vascular diabetic complications. The consequence of abnormalities, including hyperglycaemia, insulin resistance (IR), hyperinsulinaemia, and dyslipidaemia are more in T2DM patients due to increased OS. All these abnormalities contribute to overproduction of superoxide in mitochondria of endothelial cells of large and small vessels as well as the myocardium. The hyperglycaemia in diabetic complications could be explained by increased production of reactive oxygen species (ROS) through the polyol pathway flux, increased formation of advanced glycationend-products (AGEs), increased expression of the receptor for AGEs, activation of protein kinase C

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isoforms, and over-activity of the hexosamine pathway. In T2DM patients there is also inactivation of anti-atherosclerotic enzymes: endothelial nitric oxide synthase and prostacyclin synthase.[5]

In T2DM, loss of β-cell function can be due to high nutrient flux and ROS production. In muscles, liver, and heart (insulin-sensitive tissues) the oxidative damage is due to high fatty-acid flux, whereas in the eye, kidney and nervous system (non-insulin-sensitive tissues) ROS-induced diabetic complications are due to their exposure to high circulating glucose and fatty acid.[6]

There is an increased risk of premature morbidity and mortality of cardiovascular disease (CVD) in T2DM.[7] The concomitant diabetes increases the hazard associated with other risk factors for developing CVD, including hypertension, hyperlipidaemia, and renal impairment. In cardiac patients, diabetes portends an increased risk of worse outcomes in associated complications as nephropathy, retinopathy and neuropathy.[8]

Hyperglycaemia is more related to micro-vascular and metabolic complications. IR with concomitant lipid abnormalities (elevated levels of low-density lipoprotein [LDL] cholesterol, low levels of high-density lipoprotein [HDL], and elevated levels of triglyceride [TG]), thrombotic abnormalities, elevated fibrinogen with risk factors determine cardiovascular risk.[9]

Poor control of diabetes accelerates long-term complications. Thus, to prevent complications, good control of diabetes is essential and the management of diabetes should therefore aim at improving glycaemic control beyond that required to control its symptoms. There are lots of chemical agents available to control and to treat diabetic patients, but total recovery from diabetes has not been reported up to this date. In addition to adverse effects, drug treatments are not always satisfactory in maintaining euglycaemia and avoiding late-stage diabetic complications.[10]

According to WHO, 90% of the population uses plants as traditional medicine for primary healthcare purpose. The plants provide potential source of hypoglycaemic drugs and are widely used in several traditional systems of medicine to prevent diabetes. Total 800 plants have been reported to possess anti-diabetic potential.[11] Various parts of Acacia-like bark has been used to treat diarrhoea, leprosy, headache and gonorrhoea while pods and leaves have been used for treating asthma, piles, fever along with their hypoglycaemic, anti-hypolipidaemic and anti-platelets aggregation activity on diabetic animals.[12]

The current study was planned to determine the duration effect of Acacia-nilotica (AN) leaves extract and glibenclamide as hypoglycaemic, and hypolipidaemic activity in alloxan-induced diabetic rats.

Material and Methods

The experimental study was conducted at Shifa International Hospital in collaboration with National Institute of Health, Islamabad, from September 2010 to August 2011. Healthy male Sprague Dawley rats (230-250g) were housed in a group of 4 per cage. Animals were having free access to food and water ad libitum. They were kept in 12h dark/light cycle, with temperature of 25-30°C. Diabetes was induced in overnight fasted rats (10-12 hours) by alloxan. Diabetic rats of group (II to VIII) were injected intraperitonealy with 110mg/kg body weight of freshly prepared alloxan dissolved in 500 µl normal saline.[10] Control Group I received an equal quantity of citrate buffer. Rats having fasting blood glucose level of >200mg/dl on day 04 and were healthy were included in the study.

Leaves of AN, authenticated by Faculty of Biological Sciences Department of Quaid-e-Azam University, Islamabad, were mixed with 80% methanolic extract for seven days. The extract was allowed to filter through rotary evaporator under reduced pressure and then stored at -4°C

The rats were divided into 8 groups. Group I and II included the normal and diabetic control rats receiving citrate buffer [0.01M pH 4.5] AN leaves extract, 400 mg/kg b.w was given by intragastric tube as a single daily dose to group III for a period of 1 week, to group IV for 2 weeks, and to group V for a period of 3 weeks. Groups VI, VII and VIII received standard drug glibenclamide at a dose of 900 µg/kg b.w for a period of 1, 2, and 3 weeks respectively.

The rats were anaesthetised with diethyl ether and blood samples were drawn by cardiac puncture. Fasting blood glucose levels were measured on Glucometer (Optium Medisense) that works by electrical current produced by chemical reaction between glucose and glucose dehydrogenase, nicotinamide adenine dinucleotide (NAD), and phenanthelin quinine present on the glucose strip. Serum insulin was analysed on enzyme-linked immunosorbent assay (ELISA) technique. Colorimetric enzymatic method was used to measure the lipid profile. The very low density lipoprotein (VLDL) cholesterol was calculated as TG/5; while LDL was calculated by the equation; LDL cholesterol = total cholesterol - (HDL + VLDL). All estimations were done on Hitachi 911auto-analyser.

Data was statistically analysed using analysis of variance

(ANOVA) with multiple comparisons between and within the groups. Values were expressed as mean ± standard deviation and p<0.05 was considered significant.

Results

There were 64 rats in the study, with 8(12.5%) in each group. An increase (p<0.05) in the levels of fasting blood glucose, TC, TG, LDL, VLD, phospholipid (PL) and a significant decrease in serum insulin and HDL levels were observed in diabetic rats compared to normal controls (Table-1).

Induction of aqueous extract of AN leaves led to a significant decline (p<0.05) in the levels of blood glucose, TC, TG, LDL, VLDL, and rise in HDL and insulin levels in diabetic rats treated for a period of 1 week, 2 weeks and 3 weeks compared to diabetic control rats. The PL levels were non-significant (p=0.114) only when plant extract treated rats of 1 and 2 week duration were compared.

In terms of the duration effects of glibenclamide on alloxan-induced diabetic rats, a drop (p<0.05) in the levels of blood glucose, TC, TG, LDL, PL, and raise (p<0.05) in HDL levels were found in rats treated with glibenclamide for a period of 1 week compared to diabetic control group (Table-2). Similar trend (p<0.05) was also noticed when rats treated for 2 weeks were compared with those treated for 1 week, and those treated for 3 weeks compared to those treated with 2 weeks. Only the levels of VLDL in rats treated for 1 and 2 weeks (p=0.098) and the serum insulin levels between rats treated for 1 and 2 weeks (p=0.455) and treated for 2 and 3 weeks (p=0.068) were non-significant.

Discussion

The study showed the hypoglycaemic effects following oral administration of AN leaves extract in alloxan-induced diabetic rats associated with rise in serum insulin levels compared to diabetic control rats.

Our results are in agreement with earlier studies[13,14] that showed the hypoglycaemic effects of Acacia extract in diabetic rats. Our results also corroborate with a study[15] which reported that antioxidant and anti-hyperglycaemic

Table-1: One to three week plant extract treatment in Alloxan induced Diabetic rats.

Groups (n= 8)	TC (mg/dl)	TG(mg/dl)	PL(mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)	Insulin μU/ml	FBG (mg/dl)
Normal Control	78.12±2.99E	81.37±3.85E	93.00±5.39E	34.75±1.1E	16.25±.80E	59.62±4.00E	16.28± .86E	93.50±2.92 E
Diabetic control	128.12±2.90A	129.50±2.56A	144.75±5.2A	21.50±.92A	25.90±.51A	132.52±3.7A	10.53± .33A	285.0±8.07A
1wk plant treatment	106.12±3.1 B	119.50±2.44B	131.38±7.24B	26.50±1.19B	23.90±.48B	103.50±3.4B	11.84±.33B	214.12±6.7B
2wk plant treatment	98.50±2.44 C	107.62±3.6C	125.75±6.0B	31.25±1.1C	21.52±.73C	88.77±2.48C	13.11±.50 B	145.50±5.0C
3wkplant treatment	86.37±2.66D	99.50±2.4D	115.88±5.38C	34.37±1.1D	19.90±.48D	71.90±2.81D	14.12±0.54C	106.62±3.5D

Values are expressed in Means ± SEM. The different subscripts (E, A, B, C, D) used in one row show significant difference (p<0.05)
TC: Total Cholesterol
TG: Triglyceride
PL: Phospholipid
HDL-C: High density lipoprotein cholesterol
VLDL-C: Very low density lipoprotein cholesterol
LDL-C: Low density lipoprotein cholesterol
FBG: Fasting blood glucose.

Table-2: One to three week glibenclamide treatment in Alloxan induced Diabetic rats.

Groups (n=8)	TC (mg/dl)	TG(mg/dl)	PL(mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)	Insulin μU/ml	FBG (mg/dl)
Diabetic control	128.12±2.90A	129.50±2.56A	144.75±5.25A	21.50±.92 A	25.90±.51A	132.52±3.70A	10.53± .33A	285.0±8.07A
1week drug treatment	103.38±4.17 B	112.88±2.03 B	129.38±6.84 B	25.87±.83B	22.57±.40B	100.90±3.16B	14.26±.30B	217.38±6.1B
2week drug treatment	94.12±2.90C	102.12±2.35C	121.25±6.15C	30.62±1.1C	21.35±1.91B	87.32±6.92C	14.39±.36B	154.75±5.6C
3week drug treatment	82.00±2.00D	92.25±3.37 D	107.50±4.89D	34.25±.88 D	18.40±0.67 C	66.82±3.52D	14.73±.33B	113.88±5.7D

Values are expressed in Means ± SEM. The different subscripts (A, B, C, D) used in one row show significant difference (p<0.05)
TC: Total Cholesterol
TG: Triglyceride
PL: Phospholipid
HDL-C: High density lipoprotein cholesterol
VLDL-C: Very low density lipoprotein cholesterol
LDL-C: Low density lipoprotein cholesterol
FBG: Fasting blood glucose

properties of AN extract may offer a potential therapeutic source for the treatment of diabetes in alloxan-induced diabetic rats, while another study[16] showed free radical scavenging activity from leaves of AN.

Alloxan is selectively toxic to beta cells of pancreas as it preferentially accumulates in beta cells through uptake via the glucose transporter type 2 (GLUT-2). Alloxan, in the presence of intracellular thiols, generates dialuric acid in a cyclic reaction; a reduction product of ROS.

It is now known that hyperglycaemic conditions of cells are associated with the enhanced levels of ROS mainly generated by mitochondria and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. It has been established that ROS stimulates many enzymatic cascades under normal physiological conditions, but hyperglycaemia causes ROS overproduction and the deregulation of ROS signalling pathways initiating the development of DM. The toxic effect of alloxanon beta cell is initiated by formation of free radicals in redox reaction and destroys pancreatic beta cells by comprising its deoxyribonucleic acid (DNA) fragmentation.[17]

One study suggests that alloxan does not cause diabetes in humans while others found a significant difference in alloxan plasma levels in children with and without diabetes type 1.[18]

The WHO has listed more than 400 herbal plants that results in hypoglycaemia in alloxan-induced diabetic rats. According to phyto-chemical studies, hypoglycaemic effect is due to presence of tannins, and polyphenol compounds have anti-oxidant properties. The tannins restore the function of pancreatic beta cells and stimulate release of insulin, while the polyphenols reduce the blood glucose level through inhibition of α-glucosidase enzyme from the intestine. A significant decrease in blood glucose and increase in insulin levels in the treatment groups suggests antioxidant effect of the AN leaves extract which results in an increase in insulin secretion from the pancreas.[19]

Tannin is defined as "Any phenolic compound of sufficiently high molecular weight containing sufficient hydroxyls and other suitable groups (i.e. carboxyls) to form effectively strong complexes with protein and other macromolecules under the particular environmental conditions being studied."[20]

Our results are different from a study[21] that depicted the hypoglycaemic effect of AN extract in normal rabbits, but not in alloxan-induced diabetic rabbits. This might be due to the induction of AN seeds' extract instead of leaves' extract and the difference in experimental animal model. About 30-60% of the hydrophilic compounds, the tannins and polyphenols found in AN leaves results in its hypoglycaemic effects in comparison with other parts of the AN specie.[22]

Our study showed significant high levels of TG in diabetic rats compared to controls. The TG levels were decreased significantly after treatment with AN leaves' extract secondary to better glycaemic controls. Many studies had reported a decrease in lipoprotein lipase activity in DM which contributes to significant elevation of plasma TG levels, acting as risk factor for coronary heart disease. An improvement in blood glucose levels is associated with increased activity of lipoprotein lipase and a decreased plasma TG levels. Hypotriglyceridaemic effect of AN is possibly due to its cholerectic activity, which reduces the synthesis of cholesterol by hepatocytes or by decreasing its fractional reabsorption from the small intestine. These results are also in agreement with the results obtained by earlier studies[23,24] that observed that polyunsaturated fat had hypotriglyceridaemic effect.

Another study[25] showed similar results in reducing blood glucose, TG and LDL levels in AN-treated diabetic animals.

Conclusion

Acacia-nilotica leaves' extract produced hypoglycaemia and hypolipidaemia in alloxan-induced diabetic rats. The effects on diabetes increased with time duration and were comparable to drug-glibenclamide. As such, the plant may be considered effective as an alternative treatment for diabetes.

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