ORIGINAL ARTICLE

EFFECT OF *ACACIA NILOTICA* LEAVES EXTRACT ON HYPERGLYCAEMIA, LIPID PROFILE AND PLATELET AGGREGATION IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Background: To consider new hypoglycaemic, anti-hyperlipidaemic and anti-platelet aggregation sources, aqueous methanol extract of Acacia Nilotica (AN) leaves was investigated in streptozotocin induced diabetic rats. **Methods:** Diabetes mellitus was induced in 90 out of 120 male albino rats by administering 50 mg/Kg bodyweight (bw) streptozotocin intraperitonealy, and was confirmed by measuring fasting blood glucose level >200 mg/dL on 4th post-induction day. The rats were equally divided into 4 groups, A (normal control), B (diabetic control), C (diabetics rats treated with plant extract) and group D (diabetics rats treated with glyburide). The rats of group C and D were given single dose of 300 mg/Kg bw, AN extract, and 900 µg/Kg bw glyburide respectively for 3 weeks. Blood glucose levels were measured by glucometer, platelet aggregation by DiaMed method, β-thromboglobulin and insulin by ELISA technique, and lipid components were measured by enzymatic calorimetric method. **Results:** Significant differences (p<0.05) were noticed in blood glucose, serum insulin, platelet aggregation and triglyceride levels in diabetic rats treated with AN extract and glyburide as compared to diabetic controlled rats. A significant difference (p<0.05) in β-thromboglobulin and LDL levels was also noticed in rats treated with glyburide than the diabetic controlled rats. The levels of fasting blood glucose, β-thromboglobulin and platelet aggregation were significantly reduced (p<0.05) in diabetic rats treated with glyburide than AN extract treated rats. **Conclusions:** Administration of AN leaves extract showed hypoglycaemic and anti-platelet aggregation activity in diabetic rats as that of glyburide.

Keywords: Acacia Nilotica, Hypoglycemia, Streptozotocin

INTRODUCTION

Diabetes mellitus refers to a group of common metabolic disorders that share the phenotype of hyperglycaemia. Hyperglycaemia results from defect in insulin secretion, insulin action or most commonly, the both.[1] Diabetes is a global problem affecting people of all social strata throughout the world and the number of those affected is increasing day by day. According to WHO, more than 360 million people would likely to be affected by diabetes mellitus by the year 2030. In Pakistan, the number of diabetic patients is expected to increase 2 to 3 folds, from 5.2 million in the year 2000 to 13.9 million in the year 2030, making it the 4th most effected country in rank after China, India and USA.[1,2]

Diabetes mellitus is recognised by chronic hyperglycaemia and is associated with long term damage, dysfunction and failure of various body organs by involvement of micro and macro-vasculature.[3] The micro-vascular involvement mostly effects retina, renal glomeruli and peripheral nerves, while macro-vascular involvement results in dyslipidemia, formation of reactive oxygen species (ROS), advance glycation end product (AGEs), platelet hyper-reactivity and endothelial dysfunction.[4] Disturbance in endothelial function and coagulation pathway may lead to platelet activation, adhesion and aggregation.[5]

A large number of anti-diabetic medicines are available in the pharmaceutical market for diabetes and its related complications; however, currently no effective therapy is available to cure the disease. WHO Expert Committee on Diabetes has recommended investigating traditional herbal medicines[6], and in this regard more than 400 medicinal plant species have been compiled. These herbal products are gaining popularity in developing and developed countries due to their lesser side effects and low cost.[7]

The aqueous extract of AN pods, fruits, bark and seeds have been used traditionally for ailments like diarrhoea, leprosy, asthma, skin disease, ulcer, cancers of eye and ear, tuberculosis and smallpox[8]; however, its hypoglycaemic, hypolipidaemic and anti-platelets aggregation effect in diabetic animal is controversial.

The present study was designed to determine the effect of AN leaves extract on blood glucose, insulin, platelet aggregation, ß-thromboglobulin and lipid levels (total cholesterol, triglyceride, LDL and HDL) in Streptozotocin (STZ) induced diabetic rats.

MATERIAL AND METHODS

This study was conducted from November 2008 to November 2009 at the Shifa College of Medicine/Shifa International Hospital, and National Institute of Health, Islamabad on 120 healthy male albino rats weighing

225–250 gram. The rats were housed in polypropyelene cages with 12 hours dark-light cycle, at a temperature of 25–30 °C and controlled humidity of 35–60%. The animals were on free water access and standard pellet diet throughout the experiment.

In this experimental study, a total of 120 rats were divided into 4 equal groups A, B, C, and D. Group A consisted of normal rats, group B diabetic control rats, group C diabetic rats treated with AN extract and group D diabetic rats treated with glyburide.

The animals were fasted overnight (14–16 hours) before induction of diabetes by STZ. The animals of group B, C, and D were injected 50 mg/Kg bodyweight (bw) fresh STZ intra-peritonealy, prepared by dissolving in citrate buffer (0.01M, *p*H 4.5). An equal volume of citrate buffer was administered to control group A. On 4th post-treatment day, diabetes was confirmed by measuring fasting blood glucose levels. The rats who did not show fasting blood glucose levels >200 mg/dl, or showed any other symptomatic illness were excluded from the study.

Acacia Nilotica leaves were collected from the NIH Farms in Islamabad and its species was confirmed by Department of Biological Sciences Quaid-e-Azam University, Islamabad. Acacia nilotica extract was prepared in 80% aqueous methanol after crushing and macerating AN leaves, and was suspended in distilled water. The rats of group C and group D were given single morning dose of 300 mg/Kg bw AN extract and 900 µg/Kg bw glyburide respectively, by intragastric tube for 3 weeks.

Five ml of blood was drawn after 3 weeks after sacrificing the rats. Blood glucose was measured immediately by Medisense Optimum Glucometer[9]. Insulin levels were analysed by ELISA method using PRG Active Insulin ELISA reagent based on Sandwich principle. Twenty-five µL serum was mixed with the reagents and the optical density was recorded at 450 ηm with micro-titre plate reader. The insulin concentration of the samples was determined by a standard curve obtained by measuring samples of known insulin concentration.

Platelet aggregation was measured within hours by DiaMed Impact-R method using the Impact –R test reagent based on Cone and Plate principle. Five hundred µL citrated blood was mixed with 10 µL of arachidonic acid and aggregation was recorded by taking photographs.

β-thromboglobulin levels were measured within an hour by ELSIA technique using the Asserchrom BTG-reagent. The blood was mixed with anticoagulant (trisodium citrate and theophyline) and sample was allowed to cool in an ice bath. Two hundred µL of platelet free plasma was obtained by centrifugation and mixed with reagents. Optical density was read at 500 ηm with a micro-titre plate reader.

Total cholesterol, triglyceride, LDL and HDL levels were measured after mixing plasma with respective auto reagents and absorbance was measured at 500 ηm by homogeneous Enzymatic Calorimetric Test using Hitachi Auto Analyser 911.

Data was analysed using SPSS-15. Categorical variables were expressed as percentage while continuous variables were expressed as Mean±SD. Difference was considered to be significant if the Null hypothesis could be rejected with >95% confidence interval, (p<0.05 two tailed).

RESULTS

Table-1 shows comparison of fasting blood glucose, insulin, β-thromboglobulin levels and platelet aggregation between normal and experimental rats. A statistically significant elevation (p<0.05) in fasting blood glucose, β-thromboglobulin levels and platelet aggregation and a significant reduction in serum insulin levels was seen in STZ induced diabetic rats (group B) as compared to normal controls (group A).

Administration of AN leaves extract and glyburide in diabetic rats (group C and D) caused a significant reduction (p<0.05) in their fasting blood glucose levels and platelet aggregation and a significant increase (p<0.05) in serum insulin levels as compared to diabetic control rats (group B). However, a significant reduction (p<0.05) in the levels of β-thromboglobulin was observed only in rats treated with glyburide as compared to diabetic controlled rats.

When the comparison of diabetic rats treated with plant extract (group C) was done with diabetic rats treated with glyburide (group D), a significant decrease (p<0.05) was seen for fasting blood glucose, platelet aggregation and β-thromboglobulin levels, but the difference for serum insulin levels was found to be non significant (p=0.164); however, the levels in both the treatment groups remained significantly high than the control group.

Table-2 shows comparison of lipid profile (total cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL) between the normal and experimental rats. Compared with normal rats, diabetic rats showed significantly raised levels of serum cholesterol and triglyceride but change in HDL and LDL levels was found to be non significant (p=0.716, 0.052 respectively). Treatment with plant extract in diabetic rats resulted significant reduced (p<0.05) levels of triglyceride as compared to the diabetic controls, while the levels of total cholesterol (p=0.274), LDL (p=0.133) and HDL (p=0.705) were affected non-significantly. Only the levels of triglyceride and LDL in diabetic rats treated with glyburide showed a significant difference (p<0.05) from that of the diabetic controlled rats. Within the two treatment groups (C and D), the difference in the levels of total

cholesterol (p=0.437), TG (p=0.084) LDL (p=0.243) and HDL (p=0.208) were found to be non significant.

Figure-1 shows comparison of body weight between control and experimental rats. A statistically significant reduction in weight (p<0.05) was noticed in streptozotocin induced diabetic rats as compared to normal controls. The rats treated with AN extract or glyburide showed a significant increase in their body weight as compared to diabetic control rats. However, gain in weight between rats treated with plant extract and with glyburide was found to be non-significant (p=0.084).

Table-1: Comparison of fasting blood glucose, serum insulin, platelet aggregation and β-thromboglobulin levels between normal and experimental groups

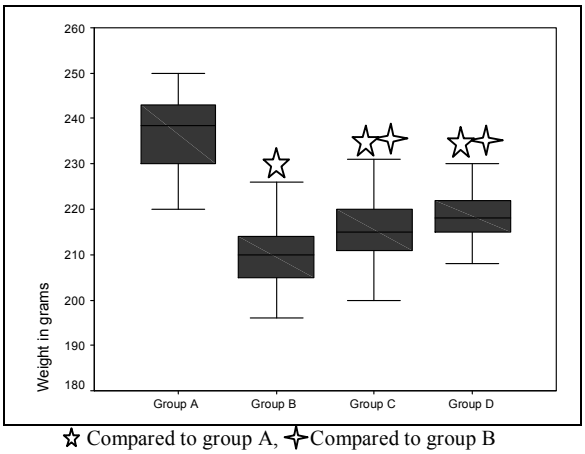
Parameters	Normal control rats Group A (n=30)	Diabetic control rats Group B (n=30)	Diabetic rats treated with plant extract Group C (n=30)	Diabetic rats treated with glyburide Group D (n=30)
Fasting blood glucose levels (mg/dL)	86.60±11.20	253.43±30.12*	162..90±26.75*†	145±23.46 *†●
Serum Insulin level (µIU/ml)	12.93±6.25	0.69±0.19*	1.48±0.77*†	1.89±1.40 *†
β-thromboglobulin level (µg/ml)	6.12±0.79	17.13±2.53*	16.26±2.72*	8.33±2.20 †●
Platelet aggregation %	2.11	8.76 *	4.35*†	3.41*†●

*Compared to group-A, †Compared to group-B, =Compared to group C

Table-2: Comparison of total cholesterol, triglyceride, high and low density lipoprotein levels between normal and experimental groups

Parameters	Normal control rats Group A (n=30)	Diabetic control rats Group B (n=30)	Diabetic rats treated with plant extract Group C (n=30)	Diabetic rats treated with glyburide Group D (n=30)
Total cholesterol mg/dL	64.80±22.31	78.70±22.50 *	73.10±16.26 *	69.87±15.74
Triglyceride mg/dL	58.33±11.31	286.00±236.29 *	123.83±18.60 *†	114.40±22.72 *†
HDL mg/dL	51.20±21.05	55.63±15.05	57.07±14.07	52.50±13.72
LDL mg/dL	11.63±6.60	8.13±3.85	9.87±4.89	11.40±5.18 †

*Compared to group A, †Compared to group B



☆ Compared to group A, ✧Compared to group B

Figure-1: Comparison of Body-weight between control and experimental rats

DISCUSSION

Our study is in consistence with a number of studies[10–12] who reported that a significant increase in fasting blood glucose, β-thromboglobulin and platelet aggregation levels and a decrease in their serum insulin levels in STZ induced diabetic rats as compared to normal controls.

Streptozotocin induces diabetes/ hyperglycaemia by changes in DNA of the pancreatic beta cells comprising its fragmentation or alkylation's.[13] Streptozotocin generates reactive oxygen species, inhibits Krebs cycle, activates poly ADP-ribose synthetase and thus depletes the intracellular NAD+ and NADP+ levels which inhibits pro-insulin synthesis and induces diabetes in a variety of animal species.[14]

In our study STZ induced diabetic rats receiving AN leaves extract and glyburide showed a significant reduction of their fasting blood glucose and an increase in serum insulin levels in comparison to diabetic control rats. The results are in consistent with Maqsood *et al*[15] who reported that AN extract significantly decreases the elevated blood glucose levels in diabetic rabbits. The results are also in corroboration with Xueqing *et al.* and Caster *et al*[16,17] who reported hypoglycemic effects of AN extract in diabetic induced animals.

More than 400 plants have been reported that effectively reduce the blood glucose levels in streptozotocin induced diabetic rats. Recent Phyto-chemical studies have shown that the hypoglycaemic effect of these plants is due to presence of tannins and polyphenols having anti-oxidant property.[18] Tannic acid possesses glucose transport-stimulatory and adipocyte differentiation–inhibitory activity and induces GLUT 4 translocation[19], while polyphenols inhibit α-glucosidase enzyme from the intestine and initiate release of insulin from the beta cells of pancreas[20,21]. Based on increased insulin levels in rats treated with AN extract and glyburide, it can be suggested that the possible mechanism of action of aqueous extract from AN could be related to anti-oxidant activity that aids to recovery from impaired glucose metabolism through release of insulin from the pancreas.

However, a study done by Wadood *et al*[22] is contradictory to our study; who showed hypoglycaemic effect of AN extract in normal rabbits but not on alloxen induced diabetic rabbits. The possible reasons may be the use of different experimental animal model and the use of Acacia seeds extract instead of Acacia leaves extract.

The hydrophilic compounds of AN, the tannins and polypherols, which are responsible for hypoglycaemic effects, are present 30–60% in the AN leaves as compared to other parts of the plant.[23]

We have shown anti-platelet aggregation effect of aqueous extract of AN leaves on the diabetic rats as compared to diabetic control rats. The results are in consistent with Gilani *et al*[24] and Rashid *et al*[25] who demonstrated anti-platelet aggregation effect of AN extract in the human subjects.

The first step in the response of platelets to vascular injury is their irreversible attachment to the altered surfaces followed by platelet aggregation. Agonists that interact with phosphoinositidase C-linked G-proteins (Gq/11) generate second messengers, inisitol tri-phosphate (IP3) and diacyl glycerol (DAG).[26] IP3 causes release of Ca^{+2} from intracellular stores (dense tubular system), and DAG activates protein kinase-C; both of these processes play a vital role in platelet aggregation in humans. The extract of Acacia nilotica inhibits platelet aggregation, produced through the blockage of Ca^{+2} influx.[27]

A number of studies[28–30] had shown significantly increased β-thromboglobulin levels in diabetic rats than the normal controls as seen in this study. The β-thromboglobulin, a low-affinity anti-heparin protein, binds to endothelial cell membrane and inhibits prostacyclin secretion. It is also regarded as useful indicator for platelet release reaction and in judging efficiency of anti-platelet therapies and prognosis.[29]

A significant increase in triglyceride (TG) levels and an increasing trend towards levels of total cholesterol in diabetic rats as compared to normal controls was shown in the present study. The results are consistent with Sochar *et al*[31] and Arkkila *et al*[32] who showed significant changes in lipid metabolism in the serum of diabetics. The increase in TG and total cholesterol levels in STZ induced diabetic rats may be due to lack of insulin under diabetic conditions.

The administration of glyburide and aqueous extract from AN effectively reduced TG levels in STZ induced diabetic rats. The results are in corroboration with Maqsood *et al*[15] who showed decreased TG levels by administration of AN extract to the diabetic animals. The decrease in TG level may be due to increased insulin release from the beta cells of pancreas that activates lipoprotein-lipase enzyme that hydrolysis TG[33]

Our study showed an increase in HDL and a decrease in LDL levels, protecting the diabetics from atherosclerotic disease probably due to control of diabetes by the AN extract as seen in a study done by Maciejewski *et al*.[34] Although abnormalities in cellular cholesterol metabolism could be partly responsible for the changes in plasma cholesterol level in diabetics, the precise mechanism underlying these enzymatic changes are yet to be elucidated.[35]

STZ is associated with a significant loss of body weight as compared to normal controls. The muscle wasting with loss and degradation of the structural proteins is probably due to hyperglycemia.[36] When these diabetic rats were treated with AN extract or glyburide, a significant gain in body weight was noticed as compared to diabetic control rats. The increase in body weight is probably due to protein anabolic effect and reversal of gluconeogenesis and glycogenolysis by the improvement of insulin secretion as a result of insulinotropic effect of AN extract and glyburide.[37] Another possible reason of increase in body weight may be the presence of 30–60% of tannins and polyphenols in the AN leaves.[23]

CONCLUSION

Acacia nilotica leaves extract produces hypoglycaemic, hypolipidemic and anti-platelet aggregation activity in streptozotocin induced diabetic rats and the effects were comparable to glyburide. Further studies are needed to investigate the active ingredients and mechanism of action of *Acacia nilotica* leaves extract for producing hypoglycaemia and anti-platelet aggregate activity.

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