

Acacia Nilotica Leave Extract and Glyburide: Comparison of Fasting Blood Glucose, Serum Insulin, β -Thromboglobulin Levels and Platelet Aggregation in Streptozotocin Induced Diabetic Rats

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Abstract

Objectives: To evaluate the hypoglycaemic and anti-platelet aggregation effect of aqueous methanol extract of Acacia Nilotica (AN) leaves compared with glyburide on streptozotocin induced diabetic rats.

Methods: Diabetes mellitus was induced in 90 out of 120 albino rats by administering 50 mg/kg body weight (b.w) streptozotocin 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 (diabetic rats treated with AN extract) and group D (diabetic rats treated with glyburide). The rats of group C and D were given 300 mg/kg b.w AN extract and 900 μ g/kg b.w glyburide respectively for 3 weeks. Blood glucose was measured by glucometer, platelet aggregation by Dia-Med method and insulin and β -thromboglobulin by ELISA technique.

Results: A significant increase ($p<0.05$) in fasting blood glucose, β -thromboglobulin and platelet aggregation and a significant decrease ($p<0.05$) in insulin levels was observed in streptozotocin induced diabetic rats than the normal controls. The rats treated with AN extract and glyburide showed a significant decrease ($p<0.05$) in fasting blood glucose and increase ($p<0.05$) in insulin levels than the diabetic control rats. However, the levels in both the treatment groups remained significantly different than the normal controls. A significant decrease ($p<0.05$) in β -thromboglobulin levels was seen in diabetic rats treated with glyburide than the diabetic control rats and diabetic rats treated with AN extract.

Conclusions: AN leaves extract result into hypoglycaemic and anti-platelet aggregation activity in diabetic rats as that of glyburide.

Keywords: Acacia Nilotica Leave Extract, Glyburide, Serum Insulin, Hypoglycaemia, Platelet Aggregation (JPMA 61:247; 2011).

Introduction

Diabetes mellitus is a syndrome with disordered metabolism and chronic hyperglycaemia due to either deficiency of insulin secretion or to a combination of insulin resistance and inadequate insulin secretion to compensate.¹

Diabetes is a global problem which has affected 171 million people in the year 2000 and the number is expected to become double by the year 2030. In Pakistan, the diabetics are expected to increase from 5.2 million in 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}

In diabetes mellitus, chronic hyperglycaemia is associated with morbidity as a result of micro and macro-vascular complications, dyslipidaemia, platelet hyper-reactivity and formation of reactive oxygen species (ROS).³ The ROS induces activation of protein kinase C, formation of advance glycation end products and endothelial dysfunction. Disturbance in endothelial function and coagulation pathway may lead to platelet activation, adhesion and aggregation⁴ by platelet agonists like Platelet Activating Factor (PAF),

epinephrine, 5-hydroxytryptamine (5HT), and Adenocinedi phosphate (ADP).⁵ A specific protein, β -thromboglobulin, stored in platelet alpha granules, is released during platelet aggregation that inhibits prostacyclin secretion and is implicated in diabetic micro-angiopathies and ischaemic and obstructive cerebro-vascular disorders.⁶

A large number of anti-diabetic medicines are available in the pharmaceutical market to reduce the ill effect of diabetes and its related complications, but no satisfactory effective therapy is available to cure the disease.⁷ WHO Expert Committee on Diabetes has recommended investigating traditional herbal medicines, and in this regard more than 400 medicinal plant species with anti-diabetic effects are compiled. These herbal products are gaining popularity in developing and developed countries due to their lesser side effects and low cost.⁸

Aqueous methanol extract from Acacia Nilotica (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;⁹ however, its hypoglycemic and anti-platelets aggregation activity on

diabetic animal is controversial.

The present study was designed to determine the effect of AN leaves extract on blood glucose, serum insulin, platelet aggregation, and beta thromboglobulin levels in Streptozotocin (STZ) induced diabetic rats.

Materials and Methods

This study was conducted [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 225-250g. The rats were housed in polypropylene cages with 12 hours dark-light cycle, at a temperature of 25-30°C and controlled humidity of 35 to 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 over night [14-16 hours] before induction of diabetes by Streptozotocin (STZ). The animals of group B, C, and D were injected intraperitoneally 50mg/kg body weight (b.w) fresh STZ, prepared by dissolving in citrate buffer [0.01M pH 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. AN 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 b.w AN extract and 900 µgm/kg b.w glyburide respectively, by intragastric tube for 3 weeks.

Five ml of blood was drawn after 3 weeks by sacrificing the rats. Blood glucose was measured immediately by Medisense Optium Glucometer¹⁰ that works by electrical current produced by chemical reaction between glucose and glucose dehydrogenase, NAD, and phenanthelin quinone present on the glucose strip.

Insulin levels were analyzed by ELISA method using PRG Active Insulin ELISA reagent based on Sandwich principle.¹¹ 25µl serum was mixed with the reagents and the

optical density was recorded at 450 nm with micro-titer 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 Dia Med Impact-R method¹² using the Impact-R test reagent based on Cone and Plate principle. 500µl citrated blood was mixed with 10µl of arachidonic acid and aggregation was recorded by taking photographs.

Beta thromboglobulin levels were measured within an hour by ELSIA technique using the Asserchrom BTG-reagent. The blood was mixed with anticoagulant (trisodium citrate and theophylline) and sample was allowed to cool in an ice bath. 200µl of platelet free plasma was obtained by centrifugation and was mixed with the reagents. Optical density was read at 500 nm with a micro-titer plate reader.

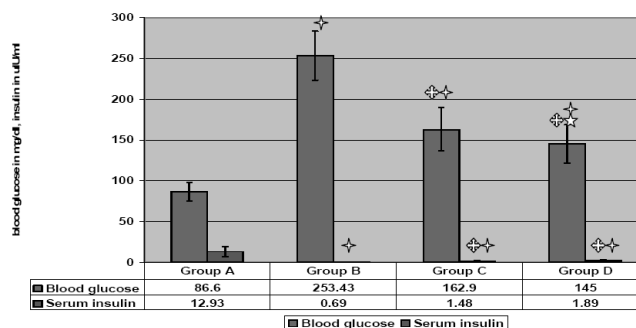
The body weight was measured by Fisher brand DP-300 weighing machine.

Statistical Analysis:

Data was analyzed using the computer software SPSS version 10.0. Categorical variables were expressed as percentage while continuous variables were expressed as mean ± S.D. Difference was considered to be significant if the Null hypothesis could be rejected > 95% confidence interval, (p<0.05 two tailed).

Results

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



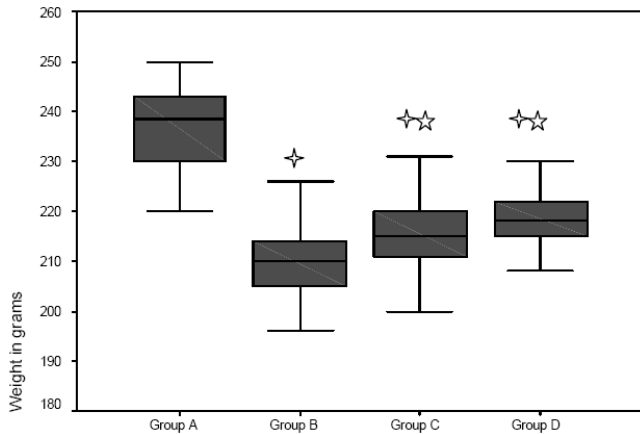
Group A: Normal control rats. Group B: Diabetic control rats. Group C: Diabetic rats treated with AN extract. Group D: Diabetic rats treated with glyburide.

✦ p < 0.05 compared to group A

✧ p < 0.05 compared to group B

☆ p < 0.05 compared to group C

Figure-1: Comparison of fasting blood glucose and serum insulin levels between normal control group and experimental groups.



Weight of groups of rats in quartiles, each box represents 100% of the values in the group, with the median as the dark middle line.
 Group A: Normal control rats. Group B: Diabetic control rats. Group C: Diabetic rats treated with plant extract. Group D: Diabetic rats treated with glyburide.
 ☆ p < 0.05 compared to group A
 ☆ p < 0.05 compared to group B

Figure-2: Comparison of Body weight between control and experimental groups.

diabetic control rats. Even after treatment the β -thromboglobulin levels remained significantly high in both the treatment groups as compared to normal controls.

A significant increase ($p < 0.05$) in the percentage of platelet aggregation was noticed in STZ induced diabetic rats than the normal controls. The percentage of platelet aggregation was significantly decreased ($p < 0.05$) in the rats treated with AN extract and glyburide than the diabetic control rats. Within the treatment groups a significant difference for platelet aggregation was seen in rats treated with glyburide as compared to rats treated with AN extract; however, even after treatment the platelet aggregation levels remained significantly high in both the treatment groups as compared to normal controls.

Figure-2 shows comparison of body weight between control and experimental rats. A statistically significant reduction in weight ($p < 0.05$) was noticed in diabetic rats as compared to normal controls. When diabetic rats were treated with AN extract or glyburide they showed a significant gain in weight as compared to diabetic control rats. However,

Table: Comparison of Beta Thromboglobulin and platelet aggregation between the experimental groups.

	Group A (n=30)	Group B (n=30)	Group C (n=30)	Group D (n=30)
β -thromboglobulin level ($\mu\text{gm/ml}$)	6.12 ± 0.79	$17.13 \pm 2.53^*$	$16.26 \pm 2.72^*$	$8.33 \pm 2.20^{\dagger o}$
Platelet aggregation %	2.11	8.76 *	4.35* \dagger	3.41* $\dagger o$

Values are described as Mean \pm S.D and percentage.

Group A: Normal control rats. Group B: Diabetic control rats. Group C: Diabetic rats treated with plant extract. Group D: Diabetic rats treated with glyburide.

*Compared to group A. \dagger Compared to group B. o Compared to group C.

Administration of AN leaves extract and glyburide in diabetic rats (group C and D) caused a significant ($p < 0.05$) reduction in fasting blood glucose and increase in serum insulin levels as compared to diabetic control rats (group B); however, the levels remained significantly different in both the treatment groups as compared to normal controls.

When the comparison of diabetic rats treated with plant extract (group C) was done with the diabetic rats treated with glyburide (group D), a significant difference ($p < 0.05$) in fasting blood glucose levels was seen but the serum insulin level was found to be non significant ($p = 0.164$).

Comparison of beta thromboglobulin and platelet aggregation between normal control and experimental groups is shown in Table. A significant increase in β -thromboglobulin was noticed in STZ induced diabetic rats as compared to the normal controls. The rats treated with glyburide showed a significant decrease in β -thromboglobulin levels as compared to diabetic control rats and diabetic rats treated with AN extract. The levels of β -thromboglobulin were found to be non significant ($p = 0.207$) when the rats treated with AN extract were compared with

when increase in weight between rats treated with plant extract and rats treated with glyburide were compared, it was found to be non significant ($p = 0.084$).

Discussion

The present study showed that STZ induced diabetic rats receiving AN leaves extract had reduction of their blood glucose and an increase in their insulin levels in comparison to diabetic control rats.

Our results are comparable with Maqsood et al¹³ who reported that AN extract decreases the elevated blood glucose levels in diabetic rabbits. The results are also in corroboration with Xueqing et al. and Caster et al^{14,15} who reported that AN extract has hypoglycaemic effect in diabetic induced animals.

Streptozotocin induces diabetes by destroying pancreatic beta cells by comprising its DNA fragmentation. The STZ generates reactive oxygen species, stimulates nuclear 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.¹⁶

The WHO has listed more than 400 herbal plants that effectively decrease the blood glucose levels in STZ induced diabetic rats. Recent Phyto-chemical studies have shown that the hypoglycaemic effect of these plants is due to presence of tannins and polyphenol compounds that have anti-oxidant properties.¹⁷ 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 β -glucosidase enzyme from the intestine.¹⁸ A significant decrease in blood glucose level and increase in insulin levels in the treatment groups suggests that the possible mechanism of action of aqueous extract from AN leaves could be anti-oxidant that aids recovery from impaired glucose metabolism through release of insulin from the pancreas.

A study done by Wadood et al¹⁹ is contradictory to our study who showed the hypoglycaemic effect of AN extract in normal rabbits but not in alloxan induced diabetic rabbits. The possible reasons may be the use 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, responsible for hypoglycaemic effects are present in leaves as compared to other parts of the AN plant.²⁰

Our results also showed anti-platelet aggregation effect of aqueous extract of AN leaves in diabetic rats as compared to diabetic control rats. The results are in consistent with Gilani et al²¹ and Rashid et al²² who demonstrated the anti-platelet aggregation effect of AN extract in normal human subject but not in the diabetics.

The first step in the response of platelets to vascular injury is their irreversible attachment to the altered surfaces followed by platelet aggregation due to the action of endogenous agonists such as arachidonic acid, adenosine diphosphate, platelet activating factor, collagen and adhesiveness of platelets to the site of injury. Agonists that interact with phosphoinositidase C-linked G-proteins (Gq/11) generate second messengers, inositol tri-phosphate (IP3) and diacyl glycerol (DAG). IP3 causes release of Ca²⁺ from intracellular stores (dense tubular system) and DAG activate protein kinase-C; both of these processes play a vital role in platelet aggregation²³ in humans. The extract of AN inhibits platelet aggregation, induced by these agonists at different potencies mediated by calcium ionophore A-23187 in a dose dependent manner, indicating the possibility of an effect produced through the blockage of Ca²⁺ influx.²⁴

The levels of β -thromboglobulin were significantly high in diabetic rats as compared to controls as shown in our study. Although treatment with AN leaves extract reduced the β -thromboglobulin levels in diabetic controls but not to the

level seen by the glyburide.

The β -thromboglobulin is a low-affinity anti-heparin protein that binds to the endothelial cell membranes and inhibits prostacyclin secretion and is regarded as a useful indicator for platelet release reaction.²⁵

In our study STZ induced diabetic rats showed a significant loss of their body weight as compared to normal control rats. The muscle wasting with loss and degradation of the structural proteins is probably due to hyperglycaemia.²⁶ 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 insulin secretion as a result of insulinotropic effect of AN extract and glyburide.⁷

Another possible reason of increase in body weight may be due to the presence of 30-60% of tannins and polyphenols in the AN leaves.¹⁸ The tannins have been used to prevent rumen degeneration of protein in lambs. 5% inclusion of tannins and polyphenols increase live weight gains by 36% and feed intake by 6%, however, at 10% inclusion, average daily gain was reduced by 18% and intake reduced by 4%.²⁷

Conclusion

Acacia nilotica leaves extract produces hypoglycaemic and anti-platelet aggregation activity in streptozotocin induced diabetic rats and the effects were comparable to glyburide; so the plant may be considered as effective and alternative treatment for diabetes.

However, further studies are needed to investigate the active ingredients of AN leaves extract and its detailed mechanism of action of producing hypoglycaemia and anti-platelet aggregator activity.

Authors would like to add that as the study has potential, it is recommended that this study should be repeated with a higher sample size (around a 50% increase).

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