HYPOGLYCEMIC AND ANTI-PLATELET AGGREGATORY EFFECT OF ACACIA MODESTA LEAVES EXTRACT ON ALLOXAN INDUCED DIABETIC RATS

MUNAZZA ASAD¹, SABEEN HAQ², TAHIR AHMAD MUNIR³, MUHAMMAD ASLAM⁴

ABSTRACT

OBJECTIVES: To compare the hypoglycemic and anti-platelet aggregation effect of Acacia modesta leaves extract and Glibenclamide on alloxan induced diabetic rats.

STUDY DESIGN: Experimental study

PLACE AND DURATION: This Experimental study was performed at Shifa College of Medicine and National Institute of Health (NIH), Islamabad.

METHODOLOGY: Diabetes mellitus was induced in 90 out of 120 male albino rats by intraperitoneal injection of 110 mg/kg bw of alloxan and was confirmed by measuring fasting blood glucose level >200 mg/dl on 4th post-induction day. The rats were equally divided into four groups, A (normal control), B (diabetic control), C (diabetic rats treated with glibenclamide), and group D (diabetic rats treated with plant extract). Rats of group C & D were treated with single dose of 900 µg/kg b.w of glibenclamide and 400 mg/kg b.w of Acacia modesta leaves extract respectively for three weeks. Blood Glucose levels were measured by glucometer, Platelet aggregation by Dia-Med and serum beta-thromboglobulin by ELIZA technique.

RESULTS: The results showed significant hypoglycemic (p<0.01) and anti-platelet aggregatory (p<0.01) effect of glibenclamide and Acacia modesta leaves extract on diabetic control rats. Within the treatment groups, the fasting blood glucose, and serum beta-thromboglobulin levels were nearly equally significant (p<0.05) in diabetic rats treated with glibenclamide and plant extract.

CONCLUSION: Acacia modesta leaves extract has a nearly equally significant hypoglycemic and anti-platelet aggregation effect on diabetic rats as that of glibenclamide.

KEYWORDS: Acacia modesta, Beta-thromboglobulin, Diabetes mellitus, Hypoglycemia, Platelet aggregation.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic syndrome characterized by derangements in carbohydrate, protein and fat metabolism associated with insulin deficiency or resistance. It is one of the most common chronic endocrine disorders contributing to morbidity and mortality worldwide. Prevalence of diabetes is growing on alarming rate. Worldwide prevalence of diabetes mellitus was 4% in 1995 and is expected to rise to 5.4% in year 2025. The major increase in diabetic patients will occur in developing countries, so by the year 2025, around 75% of diabetic patients will be residing in developing countries. According to one study, prevalence of diabetes in Pakistan was 7-11% in year 1999 and was on 7th number in WHO diabetes prevalence list. “The number of diabetic patients” in Pakistan from 4.3 million in year 1995 will rise to 14.5 million in years 2025, making Pakistan 4th highest country in the rank. Prevalence of cardiovascular disease is 3-5 times higher in diabetics as compared to non-diabetics. Several systems that maintain the integrity of vasculature are impaired in diabetes including platelet and endothelial functions, as well as processes of coagulation and fibrinolysis. Most of the morbidity and mortality seen in patients with type II diabetes mellitus is the result of micro- and macrovascular occlusive disease in which thrombosis plays an important part. Diabetic patients have lower anti thrombin III activity, protein C deficiency, increased kallikrein and increased levels of XII, XI, VIII and von willebrand factor.

Increased platelet reactivity could be due to reduction in the secretion of 2 major vasodilators, Nitric Oxide and Prostaglandins I₂ (NO and PGI₂) by the endothelium which forms the basis of endothelial abnormality in diabetes. There are data demonstrating that both glucose and insulin affect platelet aggregation, insulin stimulates NO release and increases the expression of nitric oxide synthase (NOS) by the endothelium, and that glucose may suppress this. These facts are consistent with a decrease in the ability of the blood vessels to dilate and an increase in the tendency to constrict. This imbalance contributes to the abnormalities in vascular reactivity observed in diabetes.

The important steps in thrombogenesis are; increased surface expression of platelet surface receptors such as P-selectin, GP Ib and GP Ib/IIa, possibly due to nonenzymatic glycation of the receptor proteins. GP Ib mediates binding to vWf and GP Ib/IIa binds fibrinogen. Increased activation of arachidonic acid (AA) pathway and Thromboxane A₂ (TXA₂) formation. There is more production of inositol triphosphate (IP₃) as well as increased calcium mobilization.

Clinical practice recommendations from the American Diabetes association (ADA) indicate that treatment with antiplatelet agents should be adopted for patients with diabetes with evidence of macrovascular disease, and for adults with diabetes over 30 years of age even in the absence of cardiovascular disease. Traditional use of herbs and plants is centuries old and they have shown therapeutic properties. But scientific studies are not done to confirm their efficacy and margin of Safety.

Genus Acacia is one of the very important medicinal plants, distributed in Pakistan, India and Afghanistan. Although its several species have gained importance for treatment of diabetes mellitus like, Acacia arabica, Acacia catechu, Acacia saligna, but many remain to be scientifically investigated. Acacia

1. Assistant Professor of Physiology, Al-Nafees Medical College, Islamabad
2. Assistant Professor of Physiology, Pak International Medical Institute, Peshawar
3. Associate Professor of Physiology, Rawal Institute of Health Sciences, Islamabad
4. Principal, Shifa College of Medicine & Vice Dean of Tamere-Millat University, Islamabad

Correspondence to: Dr. Munazza Asad
Assistant Professor of Physiology, Al-Nafees Medical College, Islamabad
E-Mail: munazza_wah@yahoo.com
modesta commonly known as Phulai in urdu, is a medium sized tree widely distributed in India and Pakistan. The present study has been designed to investigate the effect of Acacia modesta on platelet aggregation in animal models of Alloxan-induced diabetic rats.

**METHODOLOGY**

This Experimental study was conducted at Shifa College of Medicine, Shifa International Hospital and National Institute of Health Islamabad. The duration of the study was September 2010 to April 2011. The Aims and Objective of the study was to compare the hypoglycemic and anti-platelet aggregatory effect of Acacia modesta leaves extract and glibenclamide on alloxan induced diabetic rats. In this study, 120 healthy, active Sprague-Dawley Albino male rats, weighing 225-250 gms were included. Rats with blood sugar level < 200mg/dl after diabetes induction or having any symptomatic illness were excluded from the study. The rats were randomly divided into four groups.

### Group A
- Normal Control
- Diabetic Control (No Treatment)

### Group B
- Diabetic Rats Treated With Glibenclamide

### Group C
- Diabetic Rats Treated With Acacia Modesta

### Group D
- Diabetic Rats Treated With Acacia Modesta Leaves Extract

#### Preparation of plant extract:
The Acacia modesta leaves were collected from the farms of National Institute of Health, Islamabad and specie was authenticated by the Department of Biological Sciences at Quaid-e-Azam University, Islamabad. Its leaves were cleaned, dried and crushed into small pieces. The material was then extracted in soxhlet apparatus using 80% methanol as a solvent. After complete extraction, the solvent was evaporated and the extract was concentrated into a thick, green colored semi-solid paste under reduced pressure on a rotary vacuum evaporator and then stored at -4°C.

#### Animals:
The rats were obtained from National Institute of Health, Islamabad and were housed there in polypropylene cages, under maintained standard conditions (12 hours light and 12 hours dark cycle, 25-30°C temperature, and 35-60% humidity). The animals were fed with standard rat pellet diet and water ad libitum throughout the experiment. The animals were fasted 12 hours before the induction of diabetes but allowed free access to water.

#### Induction of Diabetes:
Diabetes was induced in overnight fasted rats of group B, C and D were kept fasted overnight but allowed free access to water. Alloxan monohydrate (110 mg/kg b.w) dissolved in 500 µl of normal saline, and a single dose of freshly prepared solution of alloxan was injected intraperitoneally. Solution was prepared separately for each rat according to its weight. Diabetes was confirmed 96 hours after alloxan administration. A drop of blood was taken from tail vein and blood glucose was measured using glucometer. Rats having blood glucose levels >200 mg/dl were included in the study.

#### Group A
- Normal control and DM was induced in groups B, C and D. The group B was taken as diabetic control group. After a week of induction, treatment was started in groups C and D for next 3 weeks. A 400 mg/kg b.w Acacia modesta leaves extract was administered to group D while 900 µg / kg b.w Glibenclamide was given to group C. Dose of plant extract was adjusted after pilot study. All treatments were given as single daily dose in the morning using intragastric tube. Equal volume of distilled water (500 µl) was given to rats of groups A and B. Two hours after last treatment, all rats (overnight fasting rats) were sacrificed under mild ether anesthesia and blood samples were collected by cardiac puncture.

#### Data collection procedure:
Five milliliters of blood was drawn by sacrificing the animals of all the groups. A drop of blood was used instantly to measure blood glucose levels with the help of glucometer. Three ml (3 ml) blood was transferred to a tube containing trisodium citrate and theophylline as anticoagulant and sample was placed immediately in ice box. Serum beta-thromboglobulin levels were determined within an hour of sample collection, using ELISA technique. One ml blood was transferred to a tube containing 3.2% trisodium citrate as anticoagulant for measuring platelet aggregation within 3 hours using Diamed Impact-R method.

#### Specific Method for Serum Beta-thromboglobulin level estimation:
All the samples (calibrator, control, plasma) were tested within 2 hours of their preparation. Specific rabbit anti-human BTG antibodies are coated on the inner side of microplate wells and the BTG to be measured bound to these antibodies. Then, anti-BTG antibodies coated with peroxidase, capture the remaining free antigenic determination of the bound BTG. Reaction is stopped after adding strong acid and the color intensity is directly proportional to BTG concentration present in the plasma sample. Optical density was read at 450 nm with a microtiter plate reader within 15 minutes after adding the stop solution.

#### Specific Method for Platelet aggregation studies:
Dia Med Impact-R is used for platelet aggregation studies. This device tests platelet aggregation in anticoagulated blood and is based on Cone and Plate principle, by placing the well under camera and photographic area covered by aggregates is measured using microtiter plate reader within 15 minutes after adding the stop solution.

### Statistical analysis:
Data was entered and analyzed on computer software SPSS version 16. The data for continuous variables was expressed as mean ± SEM. Categorical variables were expressed as percentage. Statistical analysis between the control and treated group was done by student t-test. p value < 0.05 was taken as statistically significant.

#### RESULTS:
Comparison of blood glucose levels within the study groups is shown in Table - I. The blood glucose levels in alloxan induced rats (group B) were significantly increased (p<0.05) when compared to control group (group A). A significant decrease in blood glucose levels (p< 0.05) was seen in rats treated with glibenclamide (group C) compared to diabetic controls (group B). A similar significant difference was also seen in rats treated with plant extract (group D) than those of diabetic controls (group B). When the comparison within the treatment groups was made, a significant difference (p< 0.05) was noticed in rats treated with plant extract (group D) and glibenclamide (group C).

One way ANOVA showed a statistically significant difference (p< 0.05) between the groups (A, B, C & D) when serum beta thromboglobulin levels were considered in Table - II. In group B (diabetic control) the levels of serum beta thromboglobulin were raised significantly (p<0.05) as compared to group A (normal control). While in group C (diabetic rats treated with glibenclamide) a significantly decreased levels (p<0.05) of serum beta thromboglobulin were seen as compared to group B (diabetic control). However, group D (diabetic rats treated with plant extract) showed a non-significant difference (p=0.295) as compared to group B (diabetic control).

Figure - I shows comparison of platelet aggregation within the...
four groups. Group B (diabetic control) showed significantly increased (p<0.05) platelet aggregation as compared to group A (normal control). In group C and D (diabetic treated with glibenclamide and plant extract respectively) platelet aggregation was reduced significantly (p<0.05) as compared to group B (diabetic control); however, a non-significant difference (p=0.105) was found when platelet aggregation was compared between group C and D (diabetic rats treated with glibenclamide and plant extract respectively).

Our results showed that Acacia modesta leaves extract exhibited a hypoglycemic effect in alloxan induced diabetic rats. The results are in consistence with Xueqing et al. and Caster et al. that showed hypoglycemic effects of Acacia species in diabetic induced animals. The results are also in agreement with Yasir et al. and Sunil et al. who reported that Acacia extract decreases the elevated blood glucose levels in diabetic rabbits. This fall in blood glucose level could be due to the possibility that some beta cells are still surviving to be acted upon by acacia extract to exert its effect. The phytochemical studies have shown that tannins, polyphenols and flavonoids are active ingredients of plants of genus Acacia. Tannins reduces the blood glucose levels by stimulating the transport of glucose into the cells like insulin and also causes inhibitory effect on adipocyte differentiation. They stimulate glucose transport in similar fashion like insulin. Tannic acid stimulates phosphorylation of protein factors in the insulin mediated glucose transport pathway and induces GLUT 4 translocation. Polyphenols also cause decrease in blood glucose level through inhibition of ß-glucosidase enzyme from intestine. Recent studies have shown that these polyphnolic compounds have antioxidant property which might contribute significantly to their protective effects. Flavonoids are reported to promote the beta cell regeneration. Some flavonoids have an inhibitory effect on cAMP phosphodiesterase activity that leads to stimulation of insulin secretion which reduces the blood glucose concentration.

Acacia modesta extract produce hypoglycemia in normal rats like sulphonylureas. Sulphonylureas act by closing the KATP-dependent channels, opening calcium channels, increasing intracellular calcium and accelerating insulin secretions. This suggests that mechanism of action of active ingredient of Acacia modesta resembles sulphonyluresas, mediated by enhanced insulin secretion. Ibrat et al. and Bukhari et al. reported significant anti-platelet effect of Acacia modesta leave extract in diabetic induced animals as seen in our results. The study conducted by Bukhari et al. was based on the anti-platelet aggregatory activity of Acacia nilotica in normal humans but not in diabetics. The anti-platelet aggregation activities of Acacia nilotica extract works through blockade of calcium channels. As Acacia modesta is a closely related plant of same genus, there is a possibility that Acacia modesta extract also acts through same mechanism. The immediate response of platelets to vessel wall injury is their irreversible attachment to the injured surface and release of endogenous agonists such as arachidonic acid, adenosine diphosphate, platelet activating factor, collagen that leads to platelet aggregation to the site of injury. Then there is activation of second messengers, inositol tri-phosphate (IP3) and diacyl glycerol (DAG). IP3 causes mobilization of Ca++ from intracellular stores and DAG causes activation of protein kinase-C. Hence, the AN leaves extract causes inhibition of platelet aggregation through the blockade of Ca++ influx.

Our results are in agreement with Yamada et al. who showed that plasma Beta thromboglobulin levels decreases in diabetic induced animals. Diabetics are more prone to thrombosis through complex interplay among hyperlipidemia, platelet dysfunction and endothelial injury. Platelet aggregation and

### Table I: Comparison of blood glucose levels within the study groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Post-Treatment blood Glucose (mg/dl)</th>
<th>p- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A) (n=30)</td>
<td>87.13 ± 1.62</td>
<td></td>
</tr>
<tr>
<td>Diabetic Control (B) (n=30)</td>
<td>260.33 ± 4.10</td>
<td>0.000a</td>
</tr>
<tr>
<td>Diabetics treated with glibenclamide (C) (n=30)</td>
<td>140.91 ± 3.41</td>
<td>0.000b</td>
</tr>
<tr>
<td>Diabetics treated with Acacia modesta (D) (n=30)</td>
<td>158.50 ± 3.05</td>
<td>0.000b+</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. p< 0.05 is taken as significant.

- a: Significant difference compared to normal control
- b: Significant difference compared to diabetic control
- +: Significant difference compared to rats of group C

### Table II: Comparison of serum Beta-thromboglobulin levels in all the four groups by One Way Anova

<table>
<thead>
<tr>
<th>Groups</th>
<th>Beta-thromboglobulin (µIU/ml)</th>
<th>p- Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A) (n=30)</td>
<td>6.07 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>Diabetic Control (B) (n=30)</td>
<td>13.21 ± 0.68</td>
<td>0.000a</td>
</tr>
<tr>
<td>Diabetics treated with glibenclamide (C) (n=30)</td>
<td>7.57 ± 0.35</td>
<td>0.000b</td>
</tr>
<tr>
<td>Diabetics treated with Acacia modesta (D) (n=30)</td>
<td>12.15 ± 0.73</td>
<td>0.506**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. p< 0.05 is taken as significant.

- *: Significant difference compared to controls
- **: Significant difference compared to diabetic controls
- +: Significant difference compared to normal control

### Figure I: Comparison of platelet aggregation within the groups

Values are expressed as percentage.
adhesions are increased in DM. Release of a platelet specific protein; beta-thromboglobulin is also increased from platelets of diabetic individuals. The β-thromboglobulin is a low-affinity anti-heparin protein that binds to the endothelial cell membranes and inhibits prostacyclin secretion and regarded as useful indicator for platelet release reaction.  

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