

Antidiabetic and Hypolipidemic Effects of *Aqueous Methanolic Extract of Acacia Nilotica Pods* in Alloxan-Induced Diabetic Rabbits

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Summary

This study was designed to investigate the effect of an aqueous methanol extract of *Acacia nilotica* pods (Anp) on various biochemical parameters, namely blood glucose levels, total cholesterol, High density lipids (HDLs), triglycerides, Serum Glutamate Oxaloacetate and Pyruvate Transaminase (SGOT, SGPT) and serum creatinine clearance in alloxan-induced diabetic rabbits. Rabbits were divided into three experimental groups: control, diabetic and Anp treated. The Anp treated group was further subdivided into three different groups based on the dose administered. This showed that a dose of 400 mg/kg body weight maximally reduced the blood glucose levels as compared to the diabetic group ($p < 0.001$). This dose also significantly ($p < 0.05$) lowered the plasma total cholesterol, triglyceride and Low-density lipids (LDLs) in treated rabbits as compared to diabetic rabbits. Furthermore, the same dose also significantly increased the plasma HDL levels of the treated group when compared with the diabetic group. Whereas the activity of SGOT and SGPT were decreased significantly ($p < 0.001$). Anp extract in treated diabetic rabbits. Anp treatment showed no significant effect on creatinine clearance. For interest a paper with similar aims, but using water extract of *Nigella stiva* L. appeared in this journal in 2004, (Merel *et al*, 31 (1), 49-53).

Introduction

This study was conducted to investigate the responses of alloxan-induced diabetic rabbits to an aqueous methanolic extract of Anp. Diabetes Mellitus (DM) is one of the most common metabolic disorders, with a worldwide prevalence estimated to between 1% and 5%. DM leads to abnormalities in carbohydrate, protein and lipid metabolism and increases the risk of developing atherosclerotic arterial disease by two- to six-fold (Sacks, 1997). Serum total cholesterol, triglycerides and LDL levels are increased in rabbits during the course of experimental diabetes (Maciejewski *et al.*, 2001). Diabetes mellitus also disturbs the liver

function, due to which the levels of SGOT and SGPT are increased in the blood (Chatila, 1996).

Anp has been reported to have many biological activities including antihypertensive, antispasmodic (Gilani *et al.*, 1999) and molluscicidal properties (Hussein Ayoub *et al.*, 1985). Anp are reported to be rich in tannins and polyphenols (Caster *et al.*, 1988; Kumar, 1983). Tannins such as tannic acid stimulate the transport of glucose and inhibit adipocyte differentiation (Xueqing *et al.*, 2005). Polyphenols decrease the blood glucose levels (Sabu *et al.*, 2002; Hiroshi *et al.*, 2004). Therefore, this study was designed to determine whether the aqueous methanolic extract of Anp has antidiabetic effect or not. If so, what are its effects on lipid profile, liver (SGOT and SGPT) and kidney function (Creatinine clearance) in alloxan-induced diabetic rabbits.

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Materials & Methods

Collection of Pods

Anp were collected from *Acacia nilotica* trees situated on the campus of Bahauddin Zakariya University, Multan, Pakistan. The pods were shade-dried and powdered. Prof. Dr M.H Bukhari (Ex Director Institute of Pure and Applied Biology) Department of Botany, Bahauddin Zakariya University, Multan, Pakistan authenticated the pods. A voucher specimen has been kept at the Department of Pharmacy for future reference.

Preparation of Extract

An aqueous methanolic extract of Anp was prepared using the method described by (Besra *et al.*, 2002). An aqueous methanolic extract of Anp was prepared in a Soxhlet apparatus for 8 hours using a 75:25 mixture of methanol and water. The extract was dried with the help of a rotavapor.

Animals

Male New Zealand rabbits each weighing 1-1.5 kg were used. None of the rabbits had any clinically evident infection.

Treatment of Rabbits

All animals were housed at the animal house of Department of Pharmacy, Bahauddin Zakariya University Multan. The animals were housed in stainless cages under standard laboratory condition (light period 8.00 a.m. to 8.00 p.m., 21 ± 2 °C, relative humidity 55%, green fodder and water available ad libium. The animals received humane care. The study protocol was approved by the ethical committee of the Department of Pharmacy, Bahauddin Zakariya University, Multan.

Induction of diabetes and experimental design

The rabbits were divided into three experimental groups (control, diabetic and diabetic with Anp extract treatment), each containing five rabbits. At the start of the experiment the animals in the latter two groups were injected intravenously with 80 mg/kg of 10% alloxan (Sigma Chemical Co., St

Louis, MO, USA) dissolved in isotonic saline to induce diabetes (Takahashi, 1995). The control group was injected with the same volume of isotonic saline only as the diabetic groups received. Three days after the alloxan injection, DM was confirmed by the demonstration of hyperglycemia (Blood glucose ≥ 250 mg/dl). The rabbits were not treated with insulin at any time during the experiment.

The Anp treated diabetic group was subdivided into 3 different groups: (1) receiving 200 mg/kg body weight Anp extract; (2) receiving 300 mg/kg body weight Anp extract (3) receiving 400 mg/kg body weight Anp extract every day for one month after induction of DM was confirmed. At the end of the experimental period (one month), the animals in all three groups were fasted for 12 hours (overnight) and blood samples were taken for biochemical analysis.

Biochemical analysis

The plasma glucose levels were measured, using a glucose oxidase kit (Accu check advantage II Roche Chemicals, Switzerland). The plasma total cholesterol, triglyceride, HDLs, and SGOT and SGPT levels were evaluated by enzymatic test kits (Sigma Co). The LDLs level was calculated by using the formula: $LDL = \text{total cholesterol} - HDL - (\text{triglyceride}/5)$ (Fridewald *et al.*, 1979). Creatinine clearance was determined with the help of Chemistry analyzer Microlab 200 (Merck).

Statistical analysis

The data was expressed as mean \pm standard deviation (SD) and analyzed using analysis of variance (ANOVA). Tuckey's test was used to test for differences among means for which ANOVA indicated a significant ($p \leq 0.05$) F ratio.

Results

Blood glucose level

The blood glucose concentration of diabetic rabbits 280.6 ± 7.03 mg/dl (Mean \pm SD) was increased significantly ($p \leq 0.001$) as compared to the control group at 104.2 ± 5.81 mg/dl. Treatment with the

aqueous methanolic extract of Anp significantly ($p < 0.001$) decreased the blood glucose levels of diabetic groups with mean values of 169.80 ± 11.61 , 146.60 ± 8.02 and 116 ± 3.24 mg/dl in different dose groups (200 mg/kg, 300 mg/kg, 400 mg/kg body weight Anp pods extract respectively). The blood glucose levels of the diabetic groups receiving 300 mg/kg and 400 mg/kg body weight Anp extract were within normal range (75-150 mg/dl).

Blood levels of glucose of control, diabetic and

treated groups are shown in figure 1.

Hypolipidemic effects

Cholesterol, triglyceride and LDL values increased significantly ($p \leq 0.05$) in diabetic rabbits, whereas HDL values decreased significantly ($p \leq 0.05$). Anp treatment decreased the cholesterol, triglyceride and LDL values and increased HDL values significantly ($p \leq 0.05$) in the diabetic rabbits. The lipid profile of normal, diabetic and Anp-treated diabetic rabbits is shown in Table 1.

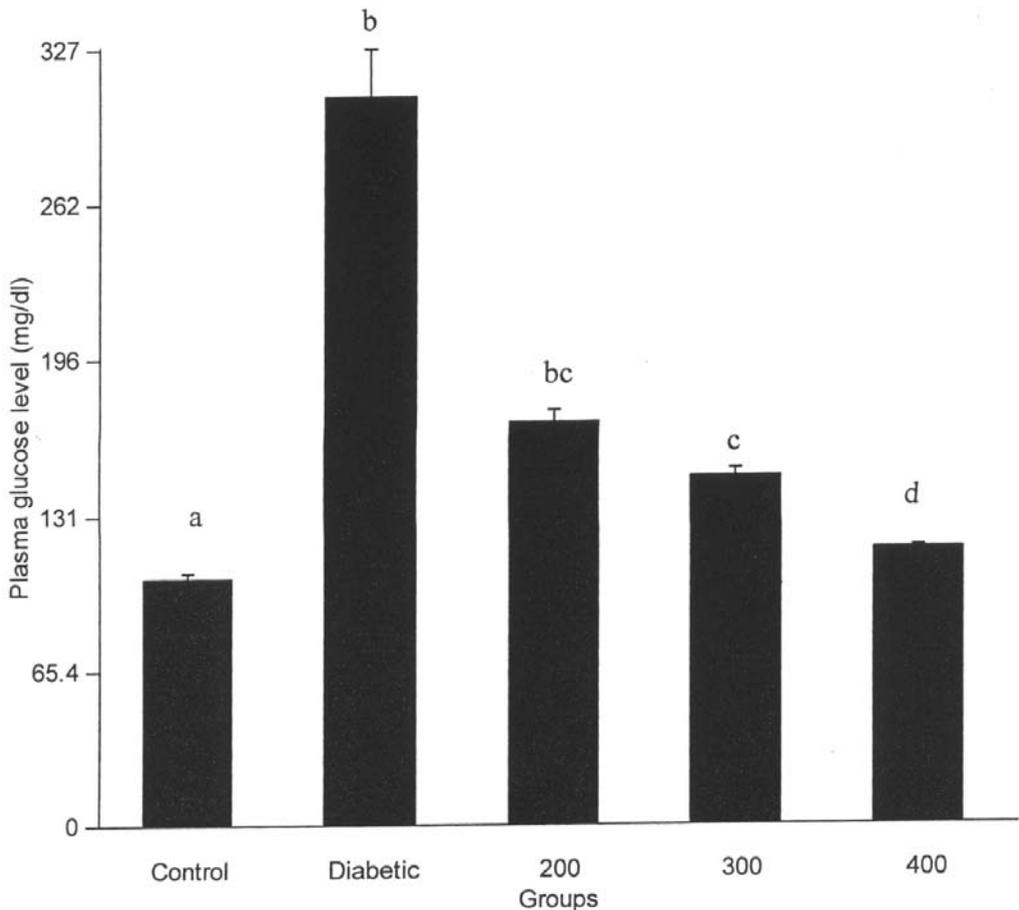


Figure 1. Effects of *Acacia nilotica* pods extract on plasma glucose levels in alloxan-induced diabetic rabbits for 1 month. Each value is mean \pm SD (Standard error mean) a, b, c, d values with different superscript letters indicate differences among the groups ($p < 0.50$, or $p > 0.001$).

Table 1. Effect of the *Acacia nilotica* pods (Anp) extract on plasma cholesterol, triglyceride, LDLs and HDLs in alloxan-induced diabetic rabbits for 1 month.

Group	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	LDLs (mg/dl)	HDLs (mg/dl)
Normal	41.4±2.07 ^a	57.6±11.80 ^a	2.28±0.955 ^a	27.6±1.14 ^a
Diabetic	59.20±3.42 ^b	137.2±30.7 ^b	9.36±7.61 ^b	21.6±2.07 ^b
Anp-treated diabetic at a dose of				
200 mg/kg	56.8 ±7.98 ^b	80.6±19.36 ^{ab}	9.28 ±3.30 ^b	29.4 ±1.51 ^a
300 mg/kg	52.8±5.36 ^c	79±18.55 ^{ab}	5.8±2.93 ^c	31.20±2.28 ^c
400 mg/kg	47.6±2.88 ^d	65.4±5.37 ^d	2.92±1.59 ^a	33.6±2.3 ^d

a, b, c, d values with different superscript letters in the same column indicate significant difference among the groups ($p < 0.05$, or $p < 0.001$). Number of rabbits in each group=5. Values are expressed as Mean±SD.

Effect on SGOT and SGPT values

SGOT and SGPT values increased significantly ($p \leq 0.05$) in diabetic rabbits. Anp extract treatment decreased ($p \leq 0.05$) the ALT and AST values in diabetic rabbits. The effects on SGOT and SGPT values in normal, diabetic and Anp-treated diabetic rabbits are shown in table 2.

Creatinine clearance

Creatinine clearance values were not significantly ($p \geq 0.05$) changed in diabetic rabbits. Table 3 shows values of creatinine clearance in control, diabetic and Anp-treated diabetic rabbits.

Discussion

DM is one of the major metabolic disorders and leads to abnormalities in lipids, carbohydrates and

Table 2. Effect of the *Acacia nilotica* pods (Anp) extract on plasma SGOT and SGPT levels in alloxan-induced diabetic rabbits for 1 month.

Group	SGOT (IU/l)	SGPT (IU/l)
Normal	153 ±15.78 ^a	160.6±9.58 ^a
Diabetic	306.6±79.6 ^b	331.6±45.8 ^b
Anp-treated diabetic, at a dose of:		
200 mg/kg	169.8±11.61 ^{ab}	199.20±18.05 ^{ab}
300 mg/kg	160.3± 9.31 ^c	182.3±12.35 ^c
400 mg/kg	155.3±13.3 ^a	172.6±30.1 ^d

a, b, c, d values with different superscript letters in the same column indicate significant difference among the groups ($p < 0.05$, or $p < 0.001$). Number of rabbits in each group=5. Values are expressed as Mean±SD

Table 3. Effect of the *Acacia nilotica* pods extract on creatinine clearance values in alloxan-induced diabetic rabbits for 1 month.

Group	Creatinine clearance
Normal	1.2±0.2
Diabetic	1.4±0.5
Anp-treated diabetic, at a dose of:	
200mg/kg	1.4±0.5
300mg/kg	1.1±0.3
400 mg/kg	1.3±0.1

Number of rabbits in each group=5

Values are expressed as Mean±SD

protein metabolism (Sacks, 1997). The present study indicated that Anp extract treatment decreases elevated glucose levels in treated diabetic rabbits. Tannins and polyphenols are reported to have antidiabetic effects (Xueqing et al., 2005; Hiroshi et al., 2004). And it is known that Anps are rich in these substances (Caster et al., 1988; Kumar, 1983). So, it can be concluded that Anp extract showed an antidiabetic effect because of the tannins and polyphenols therein.

Hypercholesterolemia and hypertriglyceridemia have been reported to occur in alloxan-induced diabetic rabbits (Maciejewski et al., 2001; Wojtowicz et al., 2004) and a significant increase observed in our experiments was in accordance with those studies. Oral administration of Anp extract significantly reduced the total cholesterol and triglyceride in plasma as compared to the diabetic group. Diabetic patients are more prone to athermatous complications such as ischemic heart disease (Batteridge, 1997; Way et al., 2001). High-density lipoprotein levels are decreased in type 2 diabetic patients and that ultimately leads to athermatous disease (HP Rang et al., 2003). It was found that oral administration of Anp extract causes an increase in HDL and decrease in LDL levels that probably protect

diabetic patients from athermatous disease. Repeated administration of Anp extract thus had a beneficial effect on the hyperlipidemia associated with hyperglycemia. The strong antihyperlipidemic activity of Anp extract could be through its control of diabetes, as this is a major determinant of triglyceride, total cholesterol and LDL levels (Laakso, 1995; Maciejewski et al., 2001).

DM is the commonest cause of liver failure and hepatomegaly (Chatila et al., 1996), which itself represents a huge and rapidly increasing problem. The increased level of SGOT & SGPT in blood indicates liver failure. This study showed that Anp treatment decreased the elevated levels of SGOT & SGPT the diabetic group. This result shows that Anp may also decrease the risk of liver failure associated with DM. Anp treatment showed no effect on kidney function and the Creatinine Clearance values were not significantly different in the three experimental groups ($p > 0.05$). The induced diabetes mellitus had no effect on the kidney function test. Chronic diabetes mellitus causes renal failure (Rang et al., 2003).

Taken together, these findings show that

- Aqueous methanolic extract of Anp causes a decrease in blood glucose levels in alloxan induced diabetic rabbits.
- Anp treatment tends to decrease the diabetes-induced rise in lipid levels and decreases the risk of athermatous disease.
- Anp treatment also improves liver function by decreasing SGOT & SGPT levels in diabetic rabbits.

Clinical investigation of Anp may enhance the chances of commercial exploitation of this extract as antidiabetic as well as hypolipidemic because these two metabolic disorders follow each other (Maciejewski et al., 2001 & Wojtowicz et al., 2004).

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