

Effect of Aqueous-Methanolic Stem Bark Extract of *Acacia polyacantha* on Blood Glucose Levels on Normoglycemic Wistar Rats

¹A.O. Okpanachi, ¹A.D.T. Goji, ¹I. Ezekiel, ²K.Y. Musa, ³Y. Tanko,
³A. Mohammed and ³A.B. Adelaiye

¹Faculty of Biomedicals, Human Physiology Department, Kampala International University, Western-Campus, Bushenyi, Uganda

²Department of Pharmacognosy,

³Department of Human Physiology, Ahmadu Bello University, Zaria, Nigeria

Abstract: This study was carried out to investigate the blood glucose reducing effect (hypoglycemic effect) of stem bark extract of *Acacia polyacantha* on normal wistar rats over a 24 h period. Three groups of previously fasted wistar rats, with each group made up of 5 rats each were administered with 100, 200 and 400 mg/kg p.o of aqueous methanolic stem bark extract of *A. polyacantha*. The hypoglycemic effect of *A. polyacantha* stem bark aqueous methanolic extract was compared with that of Metformin (250 mg/kg) in fasted normal rats. Following treatment, relatively moderate to high doses of *A. polyacantha* (100, 200 and 400 mg/kg p.o.) produced a dose-dependent, significant reduction ($p < 0.05$) in blood glucose levels of fasted normal rats. The three doses of the extract did not significantly alter the blood glucose levels after 2 h of extract administration. After 8 and 16 h of extract administration at a dose of 200 mg/kg significantly ($p < 0.05$) decreased the blood glucose levels when compared to control, while the dose of 400 mg/kg significantly decreased the blood glucose levels after 4, 8 and 16 h of extract administration when compared with the control group. The Preliminary phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, triterpenoids, anthraquinones, steroids and saponins. The median lethal dose (LD_{50}) in rats was calculated to be 3807.9 mg/kg body weight. In conclusion the aqueous methanolic extract of *Acacia polyacantha* possesses hypoglycemic activity in normoglycemic rats.

Keywords: *Acacia polyacantha*, hypoglycemic effect and phytochemical screening

INTRODUCTION

The use of herbal drugs over the years in the treatment and/or management of ailments have become widely acceptable by patients and health providers alike. The use of herbal remedies in the treatment/management of diabetes has been advocated by both orthodox and traditional health practitioners. The World Health Organization (WHO) also has thrown its support for the use of drugs of purely herbal constituents in the management and treatment of diseases (WHO, 1980). Over the centuries, herbal drugs have served as a major source of medicines for the prevention and treatment of diseases including diabetes mellitus. It is estimated that more than 500 species of plants exhibit hypoglycemic properties, including many common plants, such as pumpkin, wheat, *Mangifera indica*, lotus root and bitter melon (Handa *et al.*, 1989; Ivorra *et al.*, 1989; Jia *et al.*, 2003). Most of the plants prescribed for diabetes mellitus (DM) are not edible (Atta-ur-Rahman and Zaman, 1989; Serasinghe *et al.*, 1990) and therefore the studies on

edible plants which have a hypoglycemic effect would be of great value in the dietary management of the disease.

Acacia polyacantha also known as hook thorn or white-stem thorn is of the fabaceae family a group of plants called campylacantha (Coates Palgrave, 2002). The white-stem thorn is a large, erect tree that grows to an average height of 10-15 m, exceptionally large trees may reach a height of 25 m. The stem of younger trees appears yellowish with papery bark and persisting prickles. As it gets older, the bark gets smoother and whitish grey, with bark flakes sometimes present. Young branches are covered in silvery hairs and the whole tree is covered in dark brown to black hooked thorns in pairs.

The leaves are twice compound with 14-35 pairs of pinnae and 20-60 leaflets per pinna. Leaves are fairly large and arranged singly along the shoots. The upper surface of the leaves is darker than the underside and mostly with hairs on the margins and on the leaf stalk. The flowers that appear from September to December are light yellow to cream. The flowers as well as the seedpods are borne in spikes, which arise from the nodes. They can

appear singly or in clusters of up to four and are sweetly scented. The seed pods are flat and appear shiny green, turning to a light brown colour as they ripen and then dry out. The number of seeds per pod varies from 3 to 10 and they are also fairly flattened and dark brown when ripe (Ross, 2001). The aim of this research work is to determine the effect of aqueous ethanolic stem bark extract of *Acacia polyacantha* on blood glucose levels of normoglycemic wistar rats. This would help in contributing toward ethno-botanical uses of *Acacia polyacantha* in Nigeria and Uganda.

MATERIALS AND METHODS

Plant materials: Fresh stem bark of *Acacia polyacantha* were collected from the Ahmadu Bello University main campus and environs in the month of December 2008. It was identified and authenticated at the herbarium unit of Biological Sciences Department, A.B.U. Zaria by Mallam A.U. Gallah. It was identical with the voucher specimen (No.1905) previously deposited at the herbarium. The stem bark was dried under the shade and ground into powder.

Plant extraction: The plant extraction was carried out in April 2009 at the pharmacognosy Department, Faculty of Pharmaceutical Sciences, A.B.U. Zaria. The stem bark of *Acacia polyacantha* was air-dried and made into powder using pestle and mortar. The air-dried powdered plant (600g) material was extracted with 70% methanol and 30% aqueous (distilled water) using Soxhlet apparatus; the solvent was removed *in-vacuo* and evaporated using rotary evaporator to yield a residue of 100 g. of aqueous methanolic extract. The residue obtained was stored in a refrigerator until required for the study.

Chemicals used: All chemicals and drugs used were obtained commercially and were of analytical grade.

Phytochemical screening: The preliminary phytochemical screening of the crude extract of *Acacia polyacantha* was carried out in April 2009 by Mallam Adamu of Pharmacognosy Department, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, in order to ascertain the presence of its constituents by utilizing standard conventional protocols (Trease and Evans, 1983).

Acute toxicity study: In the first phase, 9 rats of both sexes were divided into 3 groups consisting of 3 rats each, freshly prepared crude extract of *Acacia polyacantha* was administered at doses of 10, 100 and 1000 mg/kg/orally. The animals were then monitored for signs of toxicity like; decreased locomotory activity, reduced eating,

decreased response to touch and consequently mortality for over 24 h, there was however no case of mortality after the elapse of the period. In the second phase, 3 rats were divided into 3 groups of 1 rat per group. They were administered the extract of *Acacia polyacantha* at doses of 1600, 2900 and 5000 mg/kg/orally. These groups were also monitored for 24 h with mortality recorded. Signs of toxicity were first observed 2-3 h after extract administration. These signs of toxicity observed included; convulsions, abdominal writhing, restlessness which subsequently led to insensitivity to touch and finally death. The LD₅₀ was calculated to be 3807.9 mg/kg by the log-probit using the method of Miller and Tainter (Lorke, 1983).

Animals used: Wistar strain albino rats (150-220 g) bred in the Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, A.B.U. Zaria, were used for the study. The animals were fed *ad libitum* with pellet diet (Vital feeds, Jos, Nigeria) and water. They were also maintained at room temperature in plastic cages.

In the study 25 Wistar rats weighing between (150-220 g) were used. Each group were made up of five Wistar rats (n = 5) and blood glucose levels were determined at 0, 2, 4, 8, 16 and 24 h after administration of the extract. The groupings were as follows:

- Group 1:** Received normal saline, 5 mg/kg of body weight p.o.
- Group 2:** Received 100 mg/kg body weight of the extract p.o.
- Group 3:** Received 200 mg/kg body weight of the extract p.o.
- Group 4:** Received 400 mg/kg body weight of the extract p.o.
- Group 5:** Received met for min 250 mg/kg body weight p.o.

Blood glucose levels determination: All blood samples were collected from the tail artery of the rats at intervals of 0, 2, 4, 8, 16 and 24 h. Determination of the blood glucose levels was done by the glucose-oxidase principle (Beach and Turner, 1958) using the ONE TOUCH Basic (Lifescan, Milpitas CA) instrument and results were expressed as mg/dl (Rheney and Kirk, 2000).

Statistical analysis: Blood glucose levels were expressed in mg/dl as mean ± SEM. The data were statistically analyzed using ANOVA with multiple comparisons versus control group by Dunnett's method. Values of p < 0.05 or less were considered as significant. The p value shows 95% confidence interval in our results analyzed (Duncan *et al.*, 1977).

Table 1: Showing blood glucose levels of normoglycemic Wistar rats over 24 h. after administration of aqueous methanolic extract of *Acacia polyacantha* stem bark

Groups treated	Blood Glucose Levels (mg/dl) Taken Over 24 h					
	0	2	4	8	16	24
Control (Normal Saline)	78.40±9.45	78.20±7.85	85.00±6.76	83.00±7.57	79.80±10.49	74.20±8.30
100mg/kg Extract	109.80±9.47	88.60±7.72 ^{ns}	76.00±11.44 ^{ns}	75.80±13.09 ^{ns}	78.80±8.58 ^{ns}	116.60±14.92 ^{ns}
200mg/kg Extract	93.20±4.15	92.20±4.22 ^{ns}	76.40±2.32 ^{ns}	43.00±7.57 ^a	39.11±8.58 ^a	77.40±3.83 ^{ns}
400mg/kg Extract	115.60±5.68	115.60±5.68 ^{ns}	55.00±2.17 ^a	38.80±3.98 ^a	37.60±6.71 ^a	76.40±3.83 ^{ns}
Metformin 250mg/kg	92.20±10.65	92.80±10.50 ^{ns}	97.86±10.61 ^{ns}	97.80±9.17 ^{ns}	93.20±9.98 ^{ns}	90.20±9.04 ^{ns}

Values are expressed as mean ± S.E.M; n: 5; Values considered statistically significant when compared with control group; ^a: p<0.05 significant; ^{ns}: not significant

Table 1 showed the results of the effects of three doses 100, 200 mg/Kg and 400 mg/Kg of bodyweight) of the aqueous methanolic extract of *Acacia polyacantha*, met for min and control groups in normal Wistar rats. The dose of met for min showed no significant decrease of blood glucose levels at 2, 4, 8, 16 and 24 h. The three doses of the extract did not significantly alter the blood glucose levels after 2 h. of extract administration. After 8 and 16 h of extract administration at a dose of 200 mg/kg significantly (p<0.05) decreased the blood glucose levels when compared to control, while the dose of 400 mg/kg significantly decreased the blood glucose levels after 4, 8 and 16 h. of extract administration when compared with the control group.

RESULTS

Phytochemical screening: Result of the preliminary phytochemical screening of *Acacia polyacantha* stem bark extract revealed the presence of flavonoids, steroids, carbohydrates. Tannins, anthraquinones, cardiac glycosides, alkaloids, triterpenoids and saponins.

Acute toxicity study: Signs of the toxicity were first noticed after 2-3 h. of extract administration. There was decreased locomotory activity and sensitivity to touch. Also there was decreased feed intake, tachypnea, convulsions, abdominal writhing and finally death after 6 h of extract administration.

The LD₅₀ was calculated is 3,807.9 mg/kg by the log-probit using the method of Miller and Tainter.

DISCUSSION

In this study, 3 extract treated groups (100, 200 and 400 mg/kg, respectively) were compared with a control group. The 100 mg/kg group showed no significant decrease in blood glucose level over a 24 h. period when compared with the control group. As regards to the second group which received 200 mg/kg of the extract, there was no significant change in blood glucose levels between 0-4 h. when compared with the control group. However, the 8th and 16th h showed that after extract

administration there were significant decreases (p<0.05) in blood glucose levels of the rats when compared with the normal group. By the 24th h. there was no significant change in blood glucose levels of this group when compared with the control group. The third group, which received 400 mg/kg of the extract showed no significant change in blood glucose levels after 2 h when compared with the control group. However, the 4th, 8th and 16th h. showed significant decrease (p<0.05) in blood glucose level when compared with the control group. By the 24th h. there was however no significant change in the blood glucose levels when compared with the control group. Thus, the 400 mg/kg dose was taken as the most effective dose for the study. Comparing the three groups with the met for min group reveals that the 100 mg/kg group showed no significant decrease in blood glucose levels over the 24 h. observation period. The 200 mg/kg group also showed no significant change in blood glucose level when compared with the met for min group at the 2nd and 4th. However, at the 8th and 16th h. there was significant decrease (p<0.05) in blood glucose level. The 400 mg/kg group also showed no significant change in blood glucose levels at the 2nd h when compared with the met for min group. However, by the 4th, 8th and 16th h there was significant decrease (p<0.05) in blood glucose when compared with the met for min group. By the 24th h there was no significant change in blood glucose levels in both the 200 and 400 mg/kg groups.

ACKNOWLEDGMENT:

The authors of this study wish to appreciate the technical assistance of Mallam Y'au M. of the Department of Physiology, Faculty of Medicine Ahmadu Bello University, Zaria, Nigeria, who pain-stakingly catered for the animals during the course of the experimentation.

REFERENCES

Atta-ur-Rahman and K. Zaman, 1989. Medicinal Plants with hypoglycemic activity. J Ethnopharmacol. 26(1): 1-55.

- Beach, E.F. and J.J. Turner, 1958. An enzymatic method for glucose determination in body Fluids. *Clin. Chem.*, 4: 462-468.
- Coates Palgrave, M., 2002. Keith coates palgrave trees of southern Africa . Struik Cape Town, 2: 23-25.
- Duncan, R.C., R.G. Knapp and M.C. Miller, 1977. Test of Hypotheses in Population Means, In: *Introductory Biostatistics for Health Science*. John Wiley and Son Inc., N.Y, pp: 71-76.
- Handa, S.S., A.S. Chawla and Maninder, 1989. Hypoglycaemic plants-a review. *Fitoterapia*, 60: 195-222.
- Ivorra, M.D., M. Paya and A.Villar, 1989. A review of natural products and plants as potential antidiabetic drugs. *J. Ethnopharm.*, 27: 243-275.
- Jia, W., W. Gao and L. Tang, 2003. Antidiabetic herbal drugs officially approved in China. *Phytotherapy Res.*, 17: 1127-1134.
- Lorke, D., 1983. A new approach to practical acute toxicity testing. *Archives Toxicol.*, 54: 275-287.
- Rheney, C.C. and K.K. Kirk, 2000. Performance of three blood glucose meters. *Ann pharmacother.*, March, 34(3): 317-321.
- Ross, I.A., 2001. *Medicinal Plants of the World-Chemical Constituents, Traditional and Modern Medicinal Uses*, Humana Press Inc. New Jersey, USA, 2: 30-45.
- Serasinghe, S., P. Serasinghe, H. Yamazaki, *et al.*, 1990. Oral hypoglycemic effect of *Salacia reticulata* in the streptozocin induced diabetic rat. *Phytother Res.*, 4(5): 205-295.
- Trease., G.E. and M.C. Evans, 1983. *Textbook of Pharmacognosy*, 13th Edn., Bailliere, Tindall, London, pp: 683-684
- World Health Organisation, (WHO), 1980. Second report of the WHO expert committee on diabetes mellitus. Technical report series, Ed. 646, pp: 66.