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Hypoglycaemic effects of methanol crude leaves extract and aqueous fraction of *Acacia Nilotica* on blood glucose levels on experimental animals

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ABSTRACT

The study investigated the hypoglycaemic effects of Acacia nilotica methanol crude leaves extract and aqueous fraction on blood glucose levels of Alloxan-induced diabetic wistar rats. Diabetes was induced by a single intraperitoneal (i.p) injection of Alloxan dissolved in 0.9%v/v cold normal saline solution at a dose of 150 mg/kg body weight, after which the rats were randomly divided into six groups. Group 1 served as normal control group and were administered distilled water, Group 2 received insulin (6i.u/kg), Group 3 received 500mg/kg of crude methanol extract of Acacia nilotica , Group 4 received 1000mg/kg crude methanol extract of Acacia nilotica , Group 5 received 500mg/kg aqueous fraction of Acacia nilotica and Group 6 received 1000mg/kg aqueous fraction of Acacia nilotica . All treatments were given orally for two weeks. There was a significant decrease ($P<0.05$) in the blood glucose levels when compared with the control untreated group. The phytochemical screening revealed the presences of tannins, saponins, flavonoids and steroids. The median lethal dose (LD50) in rats was calculated to be 2,154.1 mg/kg bodyweight. In conclusion, the doses of the extract has shown both significant ($p<0.05$) hypoglycemic and anti-hyperglycemic effects in Wistar rats.

Key words: Diabetes, Alloxan, *Acacia nilotica* crude extract, aqueous fraction, blood glucose levels.

INTRODUCTION

Diabetes mellitus has been considered as one of the major health concerns all around the world today [1; 2]. Diabetes mellitus is recognized 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]. Diabetes is associated with premature mortality, predominantly through atherosclerotic vascular disease [4]. Microvascular complications, which affect the small blood vessels in the eye, kidney and nerves, are associated with considerable morbidity. The economic and social costs of diabetes are enormous, both for health care services and through loss of productivity. In developed countries, 10% or more of the total health budget is spent on the management of diabetes and its complications [5].

Experimental animal models are one of the best strategies for the understanding of pathophysiology of any disease in order to design and develop the drugs for its treatment [6]. One of the most potent methods to induce experimental diabetes mellitus is chemical induction by Alloxan. It is a well- known diabetogenic agent that is used to induce Type I diabetes in experimental animals [7].

Acacia nilotica belong to the legume family and sub-family *Mimosaceae*. The common name of this tree is Babula tree, Indian gum Arabic tree. *Acacia nilotica* is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa, and in Asia eastwards to Pakistan and India. It has been introduced in China, the Northern Territory and Queensland in Australia (where it is considered to be a pest plant of national importance), in the Caribbean, Indian Ocean islands, Mauritius, United States, Central America, South America and the Galápagos Islands). It has naturalized in several countries where it has been introduced as a medicinal, forage and fuel wood plant [8].

MATERIALS AND METHODS

Plant material

The leaves of *Acacia nilotica* was collected from Ahmadu Bello University, Zaria, Nigeria. The plant material was identified and authenticated by a taxonomist, in the herbarium section in the Department of Biological Science Ahmadu Bello University, Zaria, Nigeria, where a voucher specimen (No. 698) has been deposited for future reference.

Extraction of Plant Material

The leaves of *Acacia nilotica* were air dried under the shade and grinded into free powder using morter and pestle. 200 grams of the powdered material was macerated in 100% methanol at room temperature for 24 hours. It was then filtered using a filter paper (Whatman size 1). The filtrate was then evaporated to dryness in an oven get the crude extract and kept in a sealed container at 4°C in a refrigerator until use.

Another 100 grams of the powdered material was macerated in 100% distilled water at room temperature for 24 hours. It was then filtered using filter paper (Whatman size 1). The filtrate was evaporated to dryness in an oven at 37°C. A brownish residue weighing 85grams was obtained and kept in a sealed container at 4°C in a refrigerator until use.

Chemical used

Alloxan monohydrate was purchased from Sigma chemicals (St Louis U.S.A). The Biphasic Isophane Insulin AS Mixtard 30 HM Pen fill (Novo Nordisk AIS 2880 Bagsvaerd, Denmark. NAFDAC Reg no 04-1601). Accu-chek glucometer (Lifescan, Inc 2010 Milpitas, CA 95035, U.S.A) was use for the determination of blood glucose levels.

Preliminary phytochemical screening

The fractions were subjected to preliminary phytochemical screening test for the presences of secondary metabolites according to the method described by [9] .

Acute toxicity studies (LD_{50})

Acute toxicity study: The lethal doses (LD50) of the plant extract was determined by method of [10] using 18 rats. In the first phase rats were divided into 3 groups of 3 rats each and were treated with the extract at doses of 10, 100 and 1000mg/kg body weight intraperitoneally. They were observed for 24 hours for signs of toxicity. In the second phase rats were divided into 4 groups of 3 rat each and were also treated with the methanol crude extract and the Aqueous fraction at doses of 1600, 2900 and 5000 mg/kg bodyweight (i.p).The median lethal dose (LD50) was calculated using the second phase.

Drugs and Chemicals

Chemicals used: All chemicals and drugs were obtained commercially and were of analytical grade. Alloxan monohydrate (Sigma chemical company St. Louis U.S.A).The Biphasic Isophane insulin AS Mixtard 30 HM Pen fill (Novo Nordisk AIS 2880 Bagsvaerd, Denmark. NAFDAC Reg.no 04-1601).

Experimental Animals

Animals and Induction of Diabetes Mellitus: The animals were handled in accordance with international principles guiding the use and handling of experimental animals (United State National Institute for Health, 1985). Thirty Wistar rats of both sexes weighing 120-150 g were used for the study of the effects of crude *extract* and fraction on the blood glucose levels of the animals. They were kept in standard cages at 250^{C} and 12 h light/dark condition in the animal room of the Department of Human Physiology, ABU, Zaria, Nigeria. The animals were fed on commercial feeds and were given water ad libitum .The animals were fasted from feeds for 12 hours before the

commencement of each experiment, but were allowed water *ad libitum.* The rats assigned to the diabetic groups were injected with alloxan monohydrate dissolved in sterile cold normal saline at a dose of 150 mg/kg body weight intraperitoneally as reported by earlier by [11]. Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic release of insulin, the rats were treated with 20% glucose solution intraperitoneally after 6 hours of induction of diabetes [12]. They were kept for the next 24 hours on 5% glucose solution bottles in their cages to prevent hypoglycemia. After a period of three days the rats with a blood glucose levels greater than 200mg/dl were considered diabetic were used for this research work.

Experimental Design: The alloxan -induced diabetic Wistar rats were randomly assigned into five groups (1-5) of five

Group 1: Normal control rats and received distilled water orally
Group 2: Diabetic rats were administered insulin (6 iu/kg)
Group 3: Diabetic rats were administered 500mg/kg of crude extract of *Acacia nilotica*
Group 4: Diabetic rats were administered 1000mg/kg crude extract of *Acacia nilotica*
Group 5: Diabetic rats were administered 500mg/kg aqueous fraction of *Acacia nilotica*
Group 6: Diabetic rats were administered 1000mg/kg Aqueous fraction of *Acacia nilotica* .

Determination of blood glucose levels: All blood samples were collected by cutting the tail-tip of the rats. Blood samples for blood glucose determination were collected from the tail at intervals of 1, 3, 5, 7, 9 and 12 days. Determination of the blood glucose level was done by the glucose-oxidase principle [13] using the ONE TOUCH Basic (Lifescan, Milpitas, CA) instrument and results were reported as mg/dl [14]

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 $p<0.05$ were considered as significant or less were taken as significant [15].

RESULTS

Phytochemical analysis: Freshly prepared extracts were subjected to preliminary phytochemical screening test for various constituents. This revealed the presence of alkaloids, flavonoids, saponins, steroids and tannins.

Acute toxicity study (LD50) : The sign of toxicity were first noticed after 12-18 hours of treatment. There was decreased locomotor activity and decreased in sensitivity to touch. Also there was decreased feed intake, and prostration after 12 hours of treatment. The median lethal dose (LD50) in rats was calculated to be 2,154 mg/kg body weight for both crude and aqueous fraction of the *Acacia nilotica.*

Table-1 shows comparison of blood glucose levels between control group and experimental treated rats. There was a statistically significant reduction ($p<0.05$) in blood glucose levels in alloxan induced diabetic rats treated with water fraction and aqueous leaves extract of *Acacia nilotica* as compared to the control group on day 1

Table 1: Effects of crude Methanol extract and aqueous fraction of *Acacia nilotica* on Blood glucose levels (Mean ± SEM) in alloxan-induced diabetic Wistar rats (n=5) at 12 days treatments.

Treatment	0 day	1 day	3 days	5 days	7 days	9 days	12 days
Control	483.3±10.7	445.5±60.9	578.0±13.6	578.2±33.3	483.7±40.0	425.2±79.5	446.0±20.6
Insulin 1mg/kg b.w	490.75± 7.05[a]	305.7±89.9 [ns] (37.7%)	307.7±82.2 [a] (37.3%)	119.2±59.6 [a] (75.7%)	220.2±31.2 [a] (55.1 %)	157.6±78.8 [a] (67.9%)	329.7±99.4 [ns] (32.8 %)
Crude extract 500 mg/kg b.w	486.2±22.3	234.7±46.4 [a] (51.7%)	244.0±20.8 [a] (49.8%)	177.0±27.8 (63.6 %)	167.0±18.9 (65.7 %)	201.0±24.0 (58.7 %)	174.5±28.8 (64.1%)
Crude extract 1000 mg/kg b.w	481.0±68.4	328.2±26.6 [ns] (31.7%)	279.2± 18.4 [a] (42.0%)	256.7±6.63 (46.6%)	223.7±14.5 (53.5 %)	177.7±36.7 (63.1%)	186.2±27.4 (61.3%)
Aqueous fraction 500 mg/kgb.w	486.2±22.3	200.0±31.6 [a] (50.7%)	197.5±24.8 [a] (51.3 %)	218.2± 29.5 [a] (46.2%)	229.2±57.9 [a] (43.5 %)	197.0±41.2 [a] (51.4 %)	210.0± 47.4 [a] (48.2 %)
Aqueous fraction 500 mg/kgb.w	481.0±68.4	227.0±32.4 [a] (41.8%)	157.0±42.5 [a] (59.8%)	198.2±39.0 [a] (49.2 %)	178.2± 52.5 [a] (54.3%)	205.5±39.5 [a] (47.3 %)	152.5±44.1 [a] (60.9%)

Values are expressed as mean ±SEM; n = 5

Value considered statistically when compared with control group: a = p<0.05 significant and ns = not significant

% Glycaemic change = Glucose concentration (1, 3, 5,7,9 or 12) – fasting blood glucose x 100
Fasting blood glucose

Parenthesis index ( ) means percentage (%) glycaemic change.

DISCUSSION

Alloxan monohydrate is one of the chemical agents used to induce diabetes mellitus. It induces diabetes by partial destruction of the beta-cells of Islets of Langerhan's [16].

As regards to the methanol crude extract two doses were administered that is 500mg and 1000 mg/kg b.w .. 500mg /kg of the crude extract , there was a significant decrease in the blood glucose levels ($p<0.05$) when comared with control at day 1,3,5,7,9 and 12 with percentage glycemic change of 51.7, 49.8,63.6, 65.7, 58.7, and 64.1 respectively. The highest activity resides at day 7. Also in relation to the 1000mg/kg of the methanol crude extract there was no significant change in the blood glucose levels at day 1 when compared with the control. Although at day 3, 5,7,9 and 12 there was a significant decrease ($p<0.05$) in the blood glucose levels when compared with the control with percentage glycaemic change of 42.0, 46.6, 53.5, 63.1 and 63.1 respectively. Also the highest activity resides at day 9 and 12.

In relation to the aqueous fraction two doses were administered 500 mg/kg and 1000 mg/kg b.w. As regards to the 500 mg/kg there was a significant decrease ($p<0.05$) in the blood glucose levels at day 1,3,5,7,9 and 12 when compared with the control with percentage glycaemic change of 50.7,51.3,46.2,43.5, 51.4 and 48.2 respectively. The highest activity resides at 9. Also administration of the dose of 1000mg/kg there was a significant decrease in the blood glucose levels ($p<0.05$) when compared with the control with percentage glycaemic change of 41.8,59.8 49.2, 54.3, 47.3 and 60.9 respectively. The highest activity resides at day 12. As regards to the reference drug, biphasic insulin there was significant decrease ($p<0.05$) in the blood glucose levels when compared with the control. The highest activity resides at day 5 with percentage glycaemic change of 75.7%.

The crude extract and aqueous fraction might possess Insulin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis. The phytochemical studies of extract of revealed the presence of tannins, cardiac glycosides, saponins, flavonoids. Flavonoid and tannins isolated from the other anti diabetic medicinal plants has been found to stimulate secretion or possess an insulin like-effect [17]. Effect of the flavonoids quercetin and ferulic acid on pancreatic β-cells leading to their proliferation and secretion of more insulin have been proposed by [18,19] as the mechanism by which they reduced hyperglycaemia caused by alloxan-diabetic rats. The flavonoids present in the plant may also be acting similarly thereby decreasing the high blood glucose levels of alloxan-diabetic rats. In conclusion, the results of the present study confirm the hypoglycemic effect of *Acacia nilotica* crude and aqueous fraction when administered to alloxan -induced diabetic rats, which suggest the presence of biologically active components which may be worth further investigation and elucidation. Further studies are in fact currently under way to isolate and characterize the active principle (s) of the fraction.

CONCLUSION

In conclusion, the result of the present study confirms the hypoglycaemic effect of both the aqueous fraction and crude extract of *Acacia nilotica* leaf , when administered to alloxan-induced diabetic wistar rats which suggest the presence of biologically active components responsible for the hypoglycaemic activity.

REFERENCES

[1] MW Stolar, BJ Hoogwerf, SM Gorshow, PJ Boyle, DO Wales: *J Manag Care Pharm;1*4:S2-19.
[2] DF Kruger, GM Lorenzi, G, BB Dokken, CE Sadler,K Mann, V Valentine (**2012**) *Postgraduate Medicine* **2008**;124:64-76
[3] U Hink, H Li, H Mollnau, CM Oelze, E Matheis, M Hartmann: *Circulation*; **2005** 88:14–22.
[4] World Health Organization/International Diabetes Federation: The Economics of Diabetes and Diabetes Care: **1999.** A Report of the Diabetes Health Economics Study Group. Geneva: WHO/IDF
[5] B Jonsson. **2002 ,***Diabetologia*, 45, S5-S12.
[6]A Chatzigeorgiou, A Halapas, K Kalafatakis, E Kamper .The use of animal models in the study of diabetes mellitus. *In vivo*; **2009**23:245-58.
[7] GS Viana, AC Medeiros, AM Lacerda, LK Leal, TG Vale, FJ Matos *British Medical College of Pharmacology* **2004** ;8:4-9.
[8]N Spicer, R Barnes, J Timberlake. *Indian Journal of Pharmacology;* **2007** 39:50-52.

[9]GE Trease, MC Evans Text book of Pharmacognosy 13th Edition Bailiere Tindall, London, Toronto, Tokyo.**1989** Pgs. 200-201, 340-348, 419-423, 626-630, 765-775 (**1989**).
[10]D Lork . **1983** *Achieves of Toxicology*: 275-287.
[11] B Kamewara Rao, MM Giri.,Kesavulu, CH Apparao, *Manphar Vaidhya Patrika* **1997,** 1, (1,5)33-35.
[12]A Stanley P mainzen ,MP Venugopal, *Phytother Res*.**2001** 15:213-218.
[13]EF Beach, JJ Turner. *Clin Chem.* **1958** 4: 462-468.
[14]CC Rheney,KK Kirk. *Ann Pharmacother*. **2000** March, 34 (3) 317-21.
[15]RC Duncan,RG Knapp, MC Miller. Test of hypothesis in population Means. In: Introductory Biostatistics for the health sciences. John Wiley and Sons Inc. **1977** NY pp.71-96.
[16] JA Abdel-Barry, I.A. Abdel-Hassan, M.H.H Al-Hakiem. *Journal of Ethnopharmacology.*, **1997** 58: 149-155.
[17] JR Marles,NR Farnsworth. *Phytomedicine* **1995** 2 (2)123-89.
[18] T Mahesh, PV Menon. *Phytotherapy Research*, **2004** 18: 123-127.
[19] M Sri Balasubashini, ,R Rukkumani,P Viswanathan, PV Menon. *Phytotherapy Research*, **2004** 18: 310-314.