Journal of Phytology 2009, 1(4): 260–266
ISSN: 2075-6240

Available Online: www.journal-phytology.com

REGULAR ARTICLE

HYPOGLYCAEMIC EFFECT OF THE AQUEOUS EXTRACT OF THE LEAVES OF *ALLANBLACKIA FLORIBUNDA* OLIV. (GUTTIFERAE)

Gloria A. Ayoola[1*§], Ifeoma E. Ngene[1*], Funmileyi O. Awobajo[2*], Ibiyemi I. Olatunji-Bello[4], Toluwalope O. Odugbemi[5]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Lagos, CMUL campus Idi-Araba, Lagos, Nigeria*

[2]*Deparatment of Physiology, School of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria*

[4]*Department of Physiology, College of Medicine, Lagos State University, Ikeja, Lagos, Nigeria.*

[5]*Department of Medical Microbiology, School of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria*

SUMMARY

Background: Diabetes mellitus continues to present a major medical challenge despite the array of antidiabetic medicines on the market. The disorder is characterized by chronic hyperglycaemia and glycosuria. This study aims to study the hypoglycaemic potential of the aqueous extract of *Allanblackia floribunda* leaves in alloxan-induced diabetic rats.

Methods: In the study, 25 albino rats weighing between 180-200 g were divided into five groups of five animals each. Group 1 contained normal untreated rats, group 2 contained diabetic untreated rats, group 3 & 4 were diabetic rats treated with 50 and 25 mg/kg of the aqueous extract of A. floribunda leaves respectively and group 5 contained diabetic rats treated with 0.5mg/kg glibenclamide for 15 days. Animals for the hypoglycaemic study were treated for two weeks post induction. Fasting blood glucose (FBG) levels were determined using the glucose oxidase method. FBG of the experimental animals were significantly reduced by *A. floribunda*.

Results: A 72.5% and 62.8% reduction in glucose level was observed for 50 mg/kg and 25 mg/kg dose of A. floribunda while a 71.8% reduction was noted for 0.5 mg/kg glibenclamide. Hence, 50 mg/kg dose of the aqueous extract was as potent as 0.5 mg/kg glibenclamide in inducing hypoglycaemia. Pre-treatment of the animals with the extracts for two weeks resulted in a significant lowering of the fasting blood glucose level of the animals pre-incubation and significantly reduced the effect of alloxan on FBG levels of the animals. Phytochemical screening of the aqueous leaf extract revealed the presence of tannins, flavonoids, terpenoids, saponins, cardiac glycosides, alkaloids, steroids and anthraquinones.

Conclusion: This study has demonstrated the potential of the aqueous extract of *A. floribunda* as both a hypoglycaemic agent and suggests that the extract may have a protective effect against the development of diabetes.

Keywords: *Allanblackia floribunda*, alloxan-induced, diabetic rats.

Gloria A. Ayoola et al. Hypoglycaemic effect of the aqueous extract of the leaves of *Allanblackia floribunda* Oliv. (Guttiferae). J Phytol 1 (2009) 260-266

§**Corresponding Author, *Email*:** oyetayo68@yahoo.com

1. Introduction

Diabetes mellitus is a disease characterized by chronic hyperglycaemia and glucosuria produced by an absolute or relative insufficiency of insulin. The ailment may result into the development of further metabolic and anatomic disturbances among which is lipemia, hypercholesterelaemia, loss of weight, ketosis, arteriosclerosis, gangrene, pathologic changes in the eye, neuropathy, renal disease and coma. Hyperglycaemia and glucose intolerance are common manifestations of several types of hormonal disturbances or imbalances, of which the most important is diabetes mellitus. The World Health Organization (WHO) estimates that more than 180 million people worldwide have diabetes. In 2005, an estimated 1.1 million people died from diabetes [1]. Hence despite the presence of various antidiabetic medicine on the market, diabetes and related complications continues to be a major medical problem. Urgent breakthroughs are needed in combating this disease.

The search for a cure for diabetes mellitus continues along with traditional and alternative medicine. Many herbal supplements have been used for the treatment of diabetes, but not all of them have scientific evidence to support their effectiveness [2].

Allanblackia floribunda is an evergreen tree found in the rainforest. Its traditional uses indicate possible anti-inflammatory and antimicrobial activities [3,4]. Recently, we have reported the free-radical scavenging activity and anti-inflammatory property of *Allanblackia floribunda* [5,6]. Implications of free-radicals in the pathogenesis of diabetes has prompted us to investigate the possible hypoglycaemic effect of this plant [7,8]. Hypoglycaemic effect of A. floribunda in alloxan- induced diabetic rats is hereby reported.

2. Materials and Methods

Collection and extraction of Plant sample

Allanblackia floribunda leaves were collected fresh from forest sources in Oke-igbo, Ondo State, South-West Nigeria in March 2008 by Professor TO Odugbemi. The leaves were identified at the Forestry Research Institute of Nigeria (FRIN), and given a voucher number ((FHI107929). The leaves were air dried in the oven for 3 days at a temperature of 38^{o}C and milled. The powdered leaves (300 g each) were soaked in 120 ml of distilled water for 24 h. The crude extract was filtered first through cotton wool, then through Whatman filter paper No 42 (125 mm). The extract collected was freeze-dried and stored refridgerated in a glass bottle until ready for use.

Phytochemical screening was performed using standard procedures [9,10].

Animals

Healthy adult male Sprague Dawley rats, weighing between 180-200 g were used in the present study. The animals were housed in clean cages and maintained in a well-ventilated animal house, under 12 h light and dark. The animals were fed with standard rat pellet diet (Livestock Nigeria Ltd.) and tap water was made available ad libitum. Animals were fasted before the start of the experiment. The study was carried out in accordance with the internationally accepted principles for laboratory animal use and care. Ethical approval was granted by the College of Medicine, University of Lagos, Nigeria.

Experimental design

Induction of diabetes

Rats were fasted overnight before induction of diabetes with alloxan. The rats were given an intraperitoneal injection of alloxan (160 mg/kg). Preliminary determination of the dose of alloxan

used was carried out by first treating the experimenatal animals with the usual 120 mg/kg dose of alloxan which was found to only induce diabetes in 20% of the rats induced after 48 h. Alloxan dose of 140 mg/kg induced diabetes in 50% population of rats used, while 160 mg/kg dose induced diabetes in 90% of the rats induced after 48 h. Diabetic rats used for the study were selected from the 160 mg/kg dose alloxan-treated rats. Animals with blood glucose level ≥250 mg/dl were included in the study.

Blood Sampling and Biochemical Estimation

Blood glucose level was monitored after alloxan treatment to confirm the diabetic state. Diabetes state was confirmed in the rats using glucose oxidase method using analytic kit purchased from Randox U.K. Blood samples for the glucose analysis were collected from the animals into Lithium oxalate bottles from the retro-orbital sinus using heparinised capillary tubes.

Effect of the aqueous extract of *A. floribunda* on the fasting blood glucose (FBG) levels in alloxan-induced diabetic rats

Control and diabetic rats were weighed, and divided into the following groups of five rats each

Group I (Normal Control group): They received rat feed and tap water ad libitum throughout the period of the experiment

Group II (Diabetic Control): The diabetic rats received equal volume of distilled water orally throughout the period of the experiment along with tap water and feed ad libitum.

Group III (Extract treated group 50 mg/kg): The diabetic rats were administered 50 mg/kg body weight of the aqueous leaf extract of *Allablackia floribunda* orally using oral dosing needle for 15 days.

Group IV (Extract treated group 25 mg/kg) : The diabetic rats in this group were administered 25 mg/kg body weight of the aqueous leaf extract of *Allablackia floribunda* orally using oral dosing needle for 15 days.

Group V (Reference drug group): The diabetic rats were administered a standard hypoglycaemic drug Glibenclamide obtained from May & Baker Nigeria Ltd. at a dose of 0.5mg/kg body weight for 15 days.

Effect of pre-treatment with the aqueous extract of *Allanblackia floribunda* on alloxan-induced diabetes (prohylactic study)

The animals for the prophylactic study were divided into 3 groups as follows;

Group 1: They received rat feed and tap water ad libitum throughout the period of the experiment

Group 2: The rats in this group treated with aqueous solution of extract *Allanblackia floribunda* leaves at a dose of 50 mg kg-1 body weight per day for 14 days before treatment with 160 mg/kg alloxan.

Group 3: The rats were treated with aqueous solution of extract *Allanblackia floribunda* leaves at a dose of 25 mg kg-1 body weight per day

All animals in the prophylaxis groups were treated with 160 mg/kg of alloxan after two weeks treatement with extract (Group 2 & 3) or water (Group 1).

Acute toxicity test

Thirty Swiss albino mice were divided into five goups. The extract was reconstituted in normal saline and administered orally as a single dose to groups at different concentrations (2, 4, 8, 16 and 32 g/kg). The animals were observed for a period of 48 h. The number of deaths recorded was expressed as a percentile and the LD50 was determined by probit test using the death percentage versus the log dose [11].

Statistical analysis

All data were analysed using one-way ANOVA and results presented as mean ±SEM. Level of significance was placed at P value ≤ 0.05.

3. Results and Discussion

Preliminary phytochemical screening of the extract revealed the presence of tannins, flavonoids, terpenoids, saponins, cardiac glycosides, alkaloids, steroids and anthraquinones (Table 1).

There was a significant elevation in the blood glucose level by approximately 3 times after administration of alloxan when compared to normal rats. Administration of 25 mg/kg of the aqueous extract of *A. floribunda* caused a significant and constistent reduction in the blood glucose levels of diabetic rats from day 6 onwards in a dose dependent manner. Fifty mg/kg dose of the extract was found to be as effective as 0.5 mg/kg glibenclamide in reduction of blood glucose levels in the diabetic rats (Figure 1 & Table 2).

Table 1: Phytochemical Constituents of *A. floribunda* leaves

Phytochemical Tests	floribunda leaves
Alkaloids	+
Anthraquinones	+
Cardiac glycosides	+
Flavonoids	+
Phlobatannins	-
Saponins	+
Steroids	+
Tannins	+
Terpenoids	+

Glibenclamide is a conventional antidiabetic agent that is widely used in the treatment of type 2 diabetes. A 72.5 and 71.8% reduction in blood glucose levels were observed for 50 mg/kg extract and 0.5 mg/kg glibenclamide respectively, whereas a 62.8% decrease was recorded for 25 mg/kg dose of the extract. The blood glucose level with 50 mg/kg of extract and 0.5 mg/kg glibenclamide was comparable to the levels in the normal untreated rats by day 15. However, the glucose level in the rats treated with 25 mg/kg dose were still slightly higher (97.6 mg/dl) compared to those of the normal untreated rats at day 15. There was a very great increase in blood glucose levels in the untreated diabetic rats (from 260.2 mg/dl 48 h post-induction to 450.0 mg/dl, 6 days post-induction) which resulted in death after 6 days (Figure 1 & Table 2). There was no significant change in the blood glucose levels of the control rats. A significant reduction in blood glucose levels of rats treated with the aqueous extracts pre-induction was observed (Table 3). Furthemore, pre-treatement of rats with the aqueous extracts prior to induction significantly reduced the blood glucose levels of rats post- induction compared to rats that were not pre-treated with the aqueous extract of *A. floribunda* (Table 3).

Alloxan induces diabetes through its ability to destroy the insulin secreting pancreatic β-cells resulting in a decrease in endogenous insulin release [8,12]. In vitro studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis [13]. The cytotoxic action of alloxan is mediated by reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells [14]. Hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to liver cell damage.

Table 2: Percentage reduction in fasting blood glucose level (FBG) in rats treated orally with aqueous extract of *Allablackia floribunda* for 15 days compared with non-diabetic control, and diabetic untreated and diabetic rats treated with glibenclamide rats

	Percentage reduction in fasting glucose level				
	Duration (Days)				
Group	3	6	9	12	15
Normal (Group 1)	0.00	0.82	-0.82	0.00	0.00
Diabetic control (Group2)	*-53.96a	*-73.02a	**	**	**
Diabetic+50mg/kg extract (Group 3)	20.08c,d	40.98a,c,d	60.15a,c,d	67.22a,c,d	72.56a,b,d
Diabetic + 25 mg/kg extract (Group 4)	9.24b,c,d	28.02a,b,c,d	43.05a,b,c,d	57.41a,b,c,d	62.75a,b,c,d
Diabetic + glibenclamide 0.5mg/kg (Group5)	12.91	30.15a,d	60.52a,d	68.36a,d	71.79a,d

Mean value of 5 animals per group. a= P ≤0.05 compared to the initial value, b = P ≤0.05 compared FBG for Group 3, c = P≤0.05 compared with Group 5, d =P≤0.05 compared with control (Group 1) , *increase in PBG observed with untreated diabetic rats, **death occured in animals in the untreated diabetic group. The negative sign indicates an increase in blood glucose level compare to the previous.

Table 3: Percentage increase in fasting blood glucose level in rats pretreated orally with aqueous extract of *Allanblackia floribunda* for 2 weeks before induction of diabetes with alloxan compared with rats without extract administration.

Treatment	FBG before treatment (initial-mg/dl)	FBG after treatment with extract for 2 weeks (mg/dl)	FBG 2 days after treatment with alloxan 160 mg/kg (mg/dl) (A)	% increase FBG after alloxan treatmentcompared to initial FBG
Normal (Group 1)	73.6 ± 2.4	NT	260.2 ± 6.5a	71.7
Extract (50 mg/kg – Group 2)	73.5 ± 2.7	45.5 ± 1.7a	144.5 ± 6.5a,b	49.1
Extract (25 mg/kg Group 3)	75.3 ± 2.0	65.3 ± 1.9a	193 ± 2.6a,b	61.0

Mean value of 5 animals per group. a= P≤0.05 compared to initial, b = P≤0.05 compared to normal control (Group 1). NT = Animals not treated with extract pre-induction of diabetes. The negative sign indicates a decrease in blood glucose level compare to the previous.

We have previously shown that A. floribunda possesses potent free-radical scavenging activity [5]. This may well be one of the mechanism of action of *A. floribunda*. Presence of antioxidant molecules such as flavonoids, tannins (shown in phytochemical screening experiments) in the extract may be one of the factors responsible for the observed hypoglycaemic effect of the extract [15].

Conclusion

The study has demonstrated that the aqueous extract of A. floribunda significantly reduced blood glucose levels in alloxan-induced diabetic rats and the effect of 50 mg/kg dose was comparable to that of 0.5 mg/kg glibenclamide. It was also able to prevent mortality in the diabetic rats. Furthermore, pre-treatment of the rats with the aqueous extract

prior to induction of diabetes, reduced the effect of alloxan on the blood glucose levels. This would suggest a protective effect on the pancreatic β-cells possibly by the free radical scavenging effect of the plant extracts. Furtherwork is now in progress regarding the phtyochemical(s) responsible for the hypoglycaemic effect of A. floribunda and its mechanism of action.

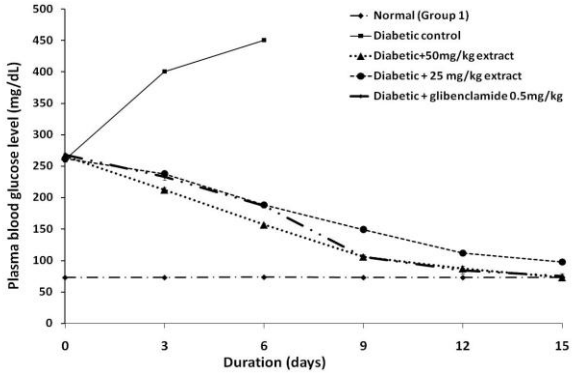

Figure 1: Graph of Plasma blood glucose level against duration of treatment. Graph shows reduction of fasting blood glucose in alloxan-induced diabetic rats by the aqueous extract of *Allanblackia floribunda* at 50 mg/kg and 25mg/kg doses. Activity at 50 mg/kg dose was comparable to that of 0.5 mg/kg glibenclamide.

LD_{50} for the aqueous extract of *Allanblackia floribunda* was determined as 9.5 g/kg. This suggests that the extract is relatively safe.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

GAA and IEN carried out plant extraction and phytochemical screening of plant material, FA and IEN carried out hypoglycaemic studies and performed the statistical analysis, IIO, TOO and CAJ participated in the design of the study, TOO carried out the plant collection, GAA concieved the study and participated in its design and co-ordination. All authors contributed to the manuscript preparation and approved the final manuscript.

Acknowledgements

We thank the University of Lagos for funding this project. We also appreciate the technical staff of the Department of Pharmaceutical Chemistry and Department of Physiology, School of Basic Medical Sciences, University of Lagos, for excellent technical support.

References

WHO:"Diabetes":Facts sheets No 312 2006, Geneva.

Ugbenyen AM, Adebimpe A: Hypoglycaemic potential of the young leaves of the methanolic extract of *Magnifera indica* in Alloxan-induced diabetic rats. Pakistan Journal of Nutrition 2009, 8(3): 239-241.

Burkill HM: The useful plants of West Tropical Africa. Royal Botanical Garden Kew.1984, 4: 385-386.

Odugbemi TO, Akinsulire O: Medicinal Plants by Species Names. In Outlines and Pictures of Medicinal Plants from Nigeria. 1st edition. Edited by Odugbemi T. University of Lagos Press, Lagos, Nigeria; 2006: pp 137.

Ayoola GA, Ipav SS, Sofidiya MO, Adepoju-Bello AA, Coker HAB, Odugbemi TO: Phytochemical Screening and Free Radical Scavenging Activities of fruits and leaves of *Allanblackia floribunda* Oliv. (Guttiferae). Int. J. of Health Res 2008, 1(2): 87-93.

Ayoola GA, Akpanika GA, Sofidiya MO, Osunkalu VO, Coker HAB, Awobajo FO, Odugbemi TO: A comparative study of the anti-inflammatory properties of *Allanblackia floribunda* and *Kigelia africana* fruits [abstract]. Planta Medica 2008, 74(9): 957.

Miller AL: Antioxidant Flavonoids: Structure, Function and Clinical Usage. Alt. Med. Rev. 1996, 1(2): 103-111.

Oberley LW: Free radicals and diabetes. Free Rad. Biol. Med 1988, 5: 113-124.

Harbone JB: Phytochemical Methods. Chapman and Hall Ltd, PA, USA. (3rd ed.), 1998.

Trease GE, Evans WC: Pharmacognosy. 13th edn. Bailliere Tindall, London, 1989.

Thompson, W.R., Weil, C.S., 1952. On the construction of tables for moving average interpolations. Biometric. 8, 51-54.

Lenzen S and Panten U: Alloxan: history and mechanism of action. Diabetologia 1988, 31: 337-342.

Jorns A, Munday R, Tiedge M, Lenzen S: Comparative toxicity of alloxan, N-alkyl-alloxans and ninhydrin to isolated pancreatic islets in vitro. J Endocrinology 1997, 155: 283-293.

Szkudelski T: The mechanism of Alloxan and streptozotocin action in β cells of the rats pancreas. Physiol Res 2001, 50: 536-46.

Loew D and Kaszkin M: Approaching the problem of bioequivalence of herbal medicinal products. Phytother. Res 2002, 16: 705-711.