Global Advanced Research Journal of Medicine and Medical Science (ISSN: 2315-5159) Vol. 3(8) pp. 186-189, August 2014
Available online http://garj.org/garjmms/index.htm

Full Length Research Paper

Phytochemical Screening and Anti-hyperglycaemic Activity of Ethanolic Extract of *Terminalia ivorensis* A. Chev. Leaves on Albino Wistar Rats

Johnny, I. I[1]*, Ekong, N. J[2] and Okon, J. E[3].

[1]Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, P.M.B. 1017, Uyo. Akwa Ibom State - Nigeria.
[2]Department of Biological Sciences, Faculty of Pure and Applied Sciences, Federal University Wukari, Taraba State, Nigeria.
[3]Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, P.M.B. 1017, Uyo. Akwa Ibom State - Nigeria.

Accepted 28 July, 2014

Phytochemical screening and anti-hyperglycaemic activity of ethanolic leaf extract of *Terminalia ivorensis* on alloxan induced wistar diabetic rats were investigated. Standard procedures were used for the evaluation. The median lethal dose (LD_{50}) of the plant was determined intraperitoneally. Results of phytochemical screening of the ethanolic extract revealed the presence of alkoloids, saponins, tannins, cardiac glycosides, terpenes and flavonoids. The LD_{50} of the leaf extract was 3464.10 mg/kg. Anti-hyperglycaemic results of the extract showed a significant ($p<0.05$) reduction in the blood glucose level (BGL) middle dose (692.82 mg/kg) at the six hours after intraperitoneal administration. The extract compared favourably with the standard drug (metformin: 100 mg/kg). This study has demonstrated the anti-hyperglycaemic potentials of *Terminalia ivorensis* leaf extract and thereby lends credence for its usage in herbal preparation for the treatment of diabetic patients in Akwa Ibom State.

Keywords: *Terminalia ivorensis*, phytochemical screening, hyperglycaemic, diabetes, albino rats, ethanolic extract, metformin.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin or cells do not respond to the insulin that is produced. There are three types of diabetes: type I which results from the body's failure to produce insulin, type II is as a result of insulin resistance and type III diabetes is gestational diabetes which occurs in pregnant women, who have never had diabetes before, but developed high blood level during pregnancy (Hopkins, 2005; Akah *et al.,* 2002). In Africa, several plants are used by traditional medicine practitioners for the treatment of diabetes. Some of these plants include *Allium sativum* (Nwaogu, 1997), and *Salvica lavandolifolia* which were found to reduce blood sugar level (Jimenez, 1995), *Dioscorea bulbifera* (Okon and Ofeni, 2013) and *Ocimum gratissimum* (Lamiaceae) whose leaf decoctions were effective oral substitutes for the control and management of hyperglycaemia (Odoemena *et al*., 2007).

*Corresponding Author E-mail: joesplendid@yahoo.com

Terminalia ivorensis (Black afara) is a large deciduous forest tree which belongs to the family Combretaceae (Hawthorne, 1995). The plant height ranges from 15 - 46 meters with small buttress and sometimes fluted roots. The branches are whorled with deciduous young shoots and foliage falling a few years after initial growth, leaving sockets to mark their original position on the bole (Adewunmi *et al.,* 2001).

Terminalia ivorensis is found in China, Guinea, Ivory Coast, Liberia, Nigeria and Serra Leone. *Terminalia* has been reported to be a large genus consisting of over 200 species of very large tress that occur extensively in the tropical regions of the World. It is usually found in forest and transition zones. The species has been successfully cultivated elsewhere in plantations (Hawthorne, 1995). The powdered bark of *Terminalia ivorensis* (commonly called black afara) is used to treat ulcers, cuts, sores and wounds (Etukudo, 2003) and as a trypanocidal agent (Adewunmi *et al*., 2001). The purpose of this study was to validate the anti-hyperglycaemic effect of *Terminalia ivorensis* leaf by traditional medicine practitioners in Akwa Ibom State.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The fresh leaves of *Terminalia ivorensis* was collected from Itak in Ikono Local Government Area, Akwa Ibom State on October, 2013. The plant was identified and authenticated by Dr. (Mrs) M. E. Bassey, plant taxonomist in Department of Botany and Ecological Studies, University of Uyo, Uyo. The voucher specimen was deposited in herbarium with number: UUH: Okon-2563.

Extraction of Terminalia ivorensis Leaves

The fresh leaves of *T. ivorensis* were shed-dried for two weeks and pulverised into fine coarse powder of uniform sizes using mortar and pestle. Five hundred grammes (500g) was macerated with 1000 ml of 70% ethanol and allowed to stand for 72 hours with occasional stirring of the solution. The solution was filtered with a plastic funnel well packed with cotton wool. The filtrate obtained was further concentrated to dryness *in vacuo* to yield 78.5g of the extract.

Collection and Maintenance of Test Animals

Healthy male and female sex albino rats weighing 150 – 250 g were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo, Uyo and maintained according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals.

Phytochemical Screening

The experiment was carried out in the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo. The phytochemical screening involves the simple chemical test to detect the presence of secondary metabolites. The methods of Trease and Evans (2009) were used for phytochemical screening.

Determination of Median Lethal Dose (LD_{50})

Swiss albino mice weighing 25-32 g were dose by the intraperitoneal (i.p.) route using the method of Lorke (1983). The animals were administered with 5000 mg/kg, 4000 mg/kg, 3000 mg/kg, 2000 mg/kg, 1000 mg/kg and 500 mg/kg of *Terminalia ivorensis* leaves extract in 5 groups of 5 mice each. The animals were observed for manifestation of physical signs of toxicity and the number of death within 24 hours was recorded. The LD_{50} was calculated as the geometric mean of the maximum dose producing 0% mortality and the minimum dose producing 100% mortality. Food was withdrawn for 18 hrs before the onset of the experiment according to methods of Amresh *et al.* (2008).

$$LD_{50} = \sqrt{D_0 \, X \, D_{100}}$$

Where: D_0 = Maximum dose producing 0% mortality

D_{100} = Minimum dose producing 100% mortality

Induction of Diabetes

Male Wistar Albino rats were made diabetic by a single dose of intraperitoneal injection of 150 mg/kg body weight of alloxan monohydrate in sterile normal saline. The rats were maintained on 5% glucose solution for next 24 hours to prevent hypoglycaemia. Four days later, blood samples were drawn from tail vein and glucose levels were determined to confirm the development of diabetes (Kim *et al.,* 2006).

EXPERIMENTAL DESIGN

Alloxan Induced Diabetic Rats

Aqueous extracts of fruits of *Terminalia ivorensis* (10 mg/ml) was orally administered to the diabetic rats at a dose of 100mg/kg after determining their initial fasting blood glucose concentration. The blood glucose

Table 1. Result of Phytochemical Screening of Ethnolic Extract of *Terminalia ivorensis*

Metabolites	Test	Observation	Inference
Alkaloids	Dragendoff's reagent	An orange precipitate was observed	++
	With Mayer's reagent	A creamy precipitate was observed	++
	With Hagger's reagent	A yellow precipitate was observed	++
Saponins	Frothing	Froth lasted for 10 minutes.	+
Tannins	Ferric chloride test	Blue-green ppt. was observed	++
Terpenes	Lieberman's Burchards test	Reddish-brown ring at interface which changed to blue-green	+
Flavonoid	Pieces of magnisum metal in acid (HCl)	Orange coloration was observed	++
Cardiac glycoside	Salkowaski's test	A brown ring at the interphase was observed	+
	Keller-Kiliani test	Reddish brown coloration at the interphase was observed	++
	Lieberman's test	Pink colouration was observed at the interphase	+++

Legend: + = slightly present, ++ = moderately present, +++ = strongly present, - = absent

Table 2. Effect of Ethanolic Leaf Extract of *Terminalia ivorensis* on Blood Glucose Level of Alloxan Induced Diabetic Rats

Groups	Treatments	0 hour	1 hour	2 hours	4 hours	6 hours
I	Metformin (100 mg/kg)	427.66±45.65*	373.33±61.22*	268.0±58.20*	139.0±28.59*	91.0±11.40*
II	Distilled water (10 mg/ml)	390.0±52.74	356.33±45.79	357.67±48.81	359.0±49.43	350.0±54.68
III	Low dose (346.41 mg/kg)	456.67±70.99*	409.33±88.72*	324.33±87.30*	274.33±67.49*	130.67±17.04*
IV	Middle dose (692.82 mg/kg)	437.27±27.22*	395.0±27.84*	303.67±25.80	210.67±20.59*	117.33±15.18*
V	High dose (1039.23 mg/kg)	430.0±31.95*	385.33±31.65*	291.0±31.75*	198.67±34.95*	104.33±19.50*

Data are processed and expressed as mean values of ± Standard Error of mean.
* Significant ($p<0.05$) when compared with the control group (n = 4).

concentration was then assayed at one hour interval for six hours (6hrs). Distilled water was administered in place of the extract for the control studies. A standard anti-diabetic drug (metformin: 100 mg/kg) was similarly administered to the diabetic rats, blood glucose concentration determined at the same interval for the same duration. The diabetic rats were divided into five groups of 5 rats each and treated as follows:

Group I: Diabetic rats treated orally with 100 mg/kg of Metformin.
Group II: Diabetic rats given 10 ml/kg of distilled water (Control).
Group III: Diabetic rats administered with 346.41 mg/kg of extract of *Terminalia ivorensis.*
Group IV: Diabetic rats administered with 692.82 mg/kg of leaf extract of *Terminalia ivorensis.*
Group V: Diabetic rats treated orally with 1039.23 mg/kg of *Terminalia ivorensis* leaf extract

For acute study, the fasting blood glucose level was monitored after 0, 1, 2, 4 and 6 hours of administration of the extract. The blood samples were collected through the tail vein just prior to and on the time frames after drug treatment. The oral glucose tolerance test (OGTT) was performed for dose of aqueous fruit extract of *Terminalia ivorensis* and blood glucose level was measured by one touch glycometer (Accu-check). The glucose level was measured at the interval of 0, 1, 2, 4 and 6 hours after the administration of the extracts.

Statistical analysis

This was carried out using windows Statistical Package for Social Sciences (SPSS) version 17.0. One way analysis of variance was adopted for comparison and the results were subject to Student's t-test using least square deviation (LSD). The data were expressed as mean ± standard error. $P<0.05$ were considered significant (Ubom, 2003).

RESULTS

The results phytochemical screening of *Terminalia ivorensis* leaf extract revealed the presence of bioactive components such as alkaloids, tannins, saponins, terpenes, flavonoids and glycosides (Table 1). The mice treated through intraperitoneal (i.p.) route with single dose of 500 - 5000 mg/kg of water soluble fraction of *Terminalia ivorensis* leaves extract showed decrease in writhing, respiratory distress, decreased limb and death.

The LD_{50} of the extract was determined to be 3464.10 mg/kg. Oral administration of water soluble fraction of *Terminalia ivorensis* at the doses of 346.41 mg/kg, 692.82 mg/kg and 1039.23 mg/kg (low, middle and high dose respectively) produced a significant ($p<0.05$) reduction in blood glucose level of alloxan induced diabetic rats, this was in dose dependent manner (Table 2). It was also observed that high dose (1039.23 mg/kg) at 6 hours of oral administration showed more patent reduction in the BGL similar to standard reference drug (metformin: 100 mg/kg) compared to control (distilled water: 10 mg/kg) which showed progressive increase (Table 2).

DISSCUSSION

The present investigation highlights the antidiabetic efficacy of ethanolic leaf extract of *Terminalia ivorensis* in alloxan induced diabetic rats treated orally with water soluble fraction (WSF) of the extract, a significant ($p<0.05$) reduction in blood glucose level (BGL) was observed. Phytochemical analysis of the extract in this study showed the present of bioactive components: alkaloids, tannins, saponins, terpenes, flavonoids and glycosides. This findings collaborates with the earlier reports of Okokon *et al.* (2009) and Odoemena *et al.* (2010) on the roles of some phytochemical components inherent in plants. Alkaloids have been reported as the active ingredient in medicinal plants exhibiting potency as antibiotic, antidiabetic and insecticidal agents (Abreu and Pereiru, 2001; Odoemena *et al*., 2007). Ahmed *et al*. (1991) stated the presence of flavonoids in plants extract effectively helped in α-cell of alloxan induced diabetic rats and reduced the blood sugar level. This could be as a result of the insulinogenic activity of flavonoids and its beneficial effect on islet of langerhan. These constituents may in part be responsible for the observed significant activity of this extract either singly or in synergy with one another. Another possible mechanism of glucose reduction utilised by the black afara (*Terminalia ivorensis*) extract through extra pancreatic mechanism by inhibition of hepatic glucose production (Swantson-Flatt *et al*., 1999; Oyedemi *et al*., 2011). The result of this research lend a credence that continuous consumption of *Terminalia ivorensis* (Black afara) leaves will always regulate and lower the blood glucose level (BGL) of a diabetic patient and it is therefore recommended as a dieting menu for hyperglycaemic patients.

CONCLUSION

This research indicated a significant anti-hyperglycaemic activity of ethanolic leaf extract of *Terminalia ivorensis* by regulating and lowering the blood glucose level (BGL) in alloxan induced diabetic rats. *T. ivorensis* is one of the natural plant remedy that is proving to be an excellent alternative in the management of diabetes mellitus.

ACKNOWLEDGMENT

The authors gratefully acknowledge the Department of Pharmacognosy and Natural Medicine and Pharmacology and Toxicology for providing laboratory space for this research. They also appreciate the technical assistance of Mr. D. N. Bala and Mr. Nsikan, M.

REFERENCES

Abreu P, Pereiru B (2001). New indole alkaloids from *Srcocephalus latifolium. Natural product Letters,* 15(1): 4-48.

Adewunmi CO, Agbedahunsi JM, Adebaja AC, Aladesanmi AJ, Murphy N, Inando J (2001). Ethnomedicine: Screening of Nigerian medicinal plants for trypanocidal properties. *J. Ethnopharmacol.,* 77: 19-24.

Ahmed F, Khalid P, Khan MM, Chanbeg M, Rastogi AK, Kidwai JR (1991). Hypoglycaemic activity of *Pterocarpus marsupium* Wood. *J. Ethnopharmacol.,* 35:71-75.

Akah PA, Okoli CO, Nwafor SV (2002). Phytotherapy in the managment of diabetes millitus. *J. Natural Remedy,* 2: 1-10.

Amresh G, Paras NS, Chandana VA (2008). Toxicological screening of traditional medicine of Laghupatha (*Cissampelos parara*) in experimental animals. *J. Ethnoparmacol*., 116: 454-460.

Etukudo I (2003). *Ethnobotany: Conventional and Traditional Uses of plants*. The Verdicts Press. Uyo, p. 41.

Hawthorne ND (1995). Ecological Profiles of Ghanalian Forest Tress. Tropical Forestry Papers 29. Oxford Forestry Institute, Department of Plant Sciences, University of Oxford, UK. 345p.

Hopkins A (2005). *Monogenic Diabetes, its Causes and Effects*. New Delhi: PVD Press, pp. 68-72.

Jimenez BJ (1995). Phytochemical screening of traditional medicine *Salvica lavandolifolia* on induced diabetic experimental animals. *J. Ethnoparmacol*., 11: 54-60.

Kim HK, Kim MJ, Cho HY, Kim EK, Shin DH (2006). Antioxidant and antidiabetic effects of amaranth (*Amaranthus esculantus*) in streptozotocin-induced diabetic rats. *Cell Biochem. Funct*., 24: 195-199.

Lorke D (1983). A New Approach to Practical Acute Toxicity testing. *Achieves of Toxicology*, 54: 278-287.

Nwaogu NK (1997). Anti-diabetic activity of aqueous leaves extract of *Alliuum sativum* in Streptozotozin induced diabetic rats. *J. Med. Plants Res*., 5(1): 119-125.

Odoemena CSI, Ekpo BAJ, Luke MI (2007). Hypoglycaemic activity of *Ocimium gratissimum* (Limm) ethanolic leaf extract mormglycaemic and hyperglycaemic rats. *J. Trop. Biosci*., 7: 24-28.

Odoemena CSI, Udosen I, Sam SM (2010). Anti-diabetic activity of *Tertracarpidium conophorum* Muell Arg. (Huteh and Dalz). *Advances in Sci. and Technol.,* 412: 120-124.

Okokon JE, Umoh EE, Etim EI, Jackson CC (2009). Anti-plasmodial and Anti-diabetic Activities of ethanolic leaf extract of *Heinsia crinata*. *J. Med. Food*, 12(1): 131-136.

Okon JE, Ofeni AA (2013). Antidiabetic Effect of *Dioscorea bulbifera* on Alloxan-induced Diabetic Rats. *ClBTech. J. Pharm. Sci.,* 2(1): 14-19.

Swantson-Flatt SK, Day C, Bailey CJ (1999). Flatt, P. R. Traditional Treatments for diabetes: Studies in normal and Streptozotozin diabetic mice, *Diabetol*., 33: 462-464.

Trease GE, Evans WO (2009). *Trease and Evans Pharmacognosy*. Sixth Edition. New York: Sauders Elsevier Limited, pp. 104-262.

Ubom RM (2004). *Biometry*. Uyo: Abaam Publishers, pp. 12 - 58.