E- ISSN: 2384-6836

International Journal of Herbs and Pharmacological Research
IJHPR, 2015, 4(2): 2 - 9
www.arpjournals.com; www.antrescentpub.com

ASN- PH-020919
ISSN: 2315-537X

RESEARCH PAPER

EVALUATION OF HYPOGLYCEMIC ACTIVITIES OF HYDROETHANOLIC LEAF EXTRACT OF *NICOTIANA TABACUM* (SOLANACEAE)

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Received: 27th March, 2015 Accepted: 21st April, 2015 Published: 30th April, 2015

***Endorsed By**: Innovative Science Research Foundation (ISREF). **Indexed By**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society*; and *IRMS Informatics India* (*J-Gate)*

ABSTRACT

The aim of this study was to evaluate the hypoglycemic activities of hydroethanolic leaf extract of *Nicotiana tabacum.* Acute toxicity was evaluated in Swiss albino mice using graded oral doses of the extract. Hypoglycemic properties of *Nicotiana tabacum* was assessed using oral glucose tolerance test and on normoglycemic rats that received single doses of the extract at 40 and 80 mg/kg body weight and blood glucose levels estimated at 2, 4 and 6 hours (single dose study). Phytochemical screening of the extract for the presence of secondary metabolites was performed with standard methods. Acute toxicity study revealed a median lethal dose (LD_{50}) of 5.82g/Kg. the single-dose study showed that 40mg/Kg and 80mg/Kg body weight of the extract significantly ($p<0.05$) reduced blood glucose levels at 2h compared to control (27.35% and 28.37% respectively), while 80mg/kg body weight of the extract significantly ($p<0.05$) reduced blood glucose level at 6h compared to control (75.40%). The oral glucose tolerance test results also showed a significant reduction ($p<0.05$) in blood glucose levels. These findings suggest that the extract of *Nicotiana tabacum* has hypoglycemic properties which may be accounted for by the presence of secondary metabolites in the extracts -flavonoids, alkaloids, phenols and terpenoids.

Key words: *Nicotiana tabacum*, Diabetes mellitus, Hypoglycemia, Oral glucose tolerance

INTRODUCTION

Diabetic mellitus is an endocrine disorder characterized by diminished production of insulin (Type 1, or Insulin-Dependent Diabetes Mellitus; IDDM) and peripheral resistance and insensitivity to insulin (Type 2, or Non-Insulin-Dependent Diabetes Mellitus; NIDDM and gestational diabetes) (Alberti *et al*., 1998; WHO, 1999). Diabetes mellitus is now recognized as one of the major killer diseases and a leading cause of death, claiming many lives worldwide (Mbaka *et al*., 2012). Nearly 7% of the world's population suffers from diabetes (Turner *et al*., 1999). It is predicted that there will be a 42% rise in diabetic patients living in the developed countries and it is likely to be close to 170% in developing countries (King *et al.*, 1998). The worldwide prevalence of diabetes mellitus was estimated in 2010 to be 285 million, and is projected to reach 439 million by 2030 (Shaw *et al.*, 2010). With this observation, the international Diabetes Federation (IDF) and World Health Organization (WHO) have declared diabetes as a global epidemic (Zimmet *et al*., 1995).

Diabetes has been conventionally treated with orthodox medicines that function as hypoglycaemic agents, or insulin production modulators and/or lipoprotein lowering agents (Ogbonnia *et al*., 2008). The high level of treatment failures, unpleasant side effects and enormous cost associated with oral antidiabetic drugs have generated an urgent need and desire for alternative treatments (Suneetha *et al.,* 2010). The preferred choice of plant medicine by many might not be unconnected with the historical successes recorded in the use of herbal product in traditional system of medicine in managing diabetes mellitus (Mbaka *et al.,* 2012). Herbal formulations were observed to have fewer side effects and less toxic because of their rich natural source. Based on these and the support provided for its practice by the World Health Organization, several scientific investigations are being conducted with the view of identifying new active ingredient of natural source that would be more effective in the treatment of diabetes mellitus and diabetic complications (WHO, 1980).

The utilisation of plants and their derivatives for the treatment and/or management of various diseases, including diabetes mellitus (DM), are becoming more and more prominent in pharmaceutical markets as an alternative and/or complementary therapy. *Nicotiana tabacum* is one of those plants used traditionally in the treatment of diabetes mellitus. *Nicotiana tabacum* is a herbaceous plant that belongs to the family Solanaceae. It originated in the tropical Americas (South America, Mexico and West Indies) and now cultivated world wide as the commercial source of tobacco. *Nicotiana tabacum* has been reported to have antibacterial activities (Barkht *et al.,* 2012) and has also been shown to exhibit antifungal activities (Sela-Buurlage *et al.,* 1993). Studies have shown that people with alzheimers disease may benefit from nicotine, a natural alkaloid in tobacco (Ksir *et al*., 1983, Whitehouse *et al*., 1986, Wilson *et al.,* 1995). Epidemiological studies have also demonstrated an inverse relation between smoking and the development of Parkinson's disease, with an odds ratio of about 0.5 for smokers compared with nonsmokers (smokers are half as likely to have Parkinson's disease) (Baron 1986, Tzourio *et al.*, 1997).

Unfortunately, barely any scientific study has been conducted on the antidiabetic activity of this plant. Of course, that tobacco has numerous side effects like various types of cancer, heart attack, stroke, atherosclerosis, chronic obstructive pulmonary disease, etc, is not in doubt, but surely, most of its beneficial effects have not been inadvertently ignored and unexplored. This study therefore, evaluates the hypoglycaemic activities of hydroethanolic leaf extract of *Nicotiana tabacum*.

MATERIALS AND METHODS

Plant Materials: The fresh leaves of *Nicotiana tabacum* were obtained from a farm in Uromi, Edo State, Nigeria. They were authenticated by a taxonomist, Mr T.K Odewo, of Botany department, University of Lagos. The voucher specimen with number LUH 1203 was deposited in the University herbarium.

Preparation of the Plant Material for Extraction: The fresh leaves were washed with clean water to remove foreign materials, chopped into small pieces and dried in an oven at 45 degrees centigrade for 4 days. The dried leaves were blended to fine particles with an electric blender. The leaf powder, weighing 200g, was extracted with 90% hydro- ethanol by maceration with frequent stirring for 5days. The extract was filtered using Watman filter paper 4 and concentrated with a rotary evaporator. The concentrated extract was dried in an oven at 40 degrees centigrade to obtain 14g dry residue (7% yields)

Animals: Swiss mice (20 - 25g) and Wistar rats (160±20g) of both sexes were obtained from the Laboratory Animal house of the College of Medicine, University of Lagos, Idi-Araba and kept under standard environmental conditions (12/12hr light/dark cycle). They were housed in cages (5 animals per cage), maintained on standard animal pellets (Pfizer Feeds Plc, Nigeria), and provided with water *ad libitum*. They were allowed to acclimatize for seven days to the laboratory conditions before the experiment. The use and care of the animals, were in strict compliance with the National Research Council guidelines on the care and use of laboratory animals (NRC, 2011).

Acute Toxicity Study: The toxicity study was carried out using thirty five (35) (male and female) Swiss albino mice weighing between 20 – 25g. The animals were randomly distributed into a control group and six treated groups, consisting of five animals per group. After fasting the animals overnight, the control group was given 0.4ml Acacia (2%) suspension orally. while each of the treatment groups received oral solution of the extract prepared with 2% acacia in the doses of 1.0, 2.5, 5.0, 10.0, and 15.0 and 20.0 g/kg body weight respectively. The animals were observed continuously for the first 4 hours and then hourly for the next 24hours and at 6 hourly interval for the next 48 hours after administering the extract to observe any death or changes in general behaviour and other physiological activities (Shah *et al*., 1997, Bürger *et al.*, 2005). The dose that results in 50% mortality (LD_{50}) was then determined.

Phytochemical screening: Phytochemical screening of the extract for the presence of secondary metabolites was performed with standard methods using the following reagents and chemicals: alkaloids with Mayer's reagent and Dragendorff's reagent (Farnsworth,1966; Harborne, 1998), flavonoids with the use of 10% lead acetate and 20% sodium hydroxide (Sofowora 1993, Trease and Evans 1996), phenols and tannins with 2% ferric chloride solution (Yadav and Agarwala, 2011) and saponins with ability to produce suds (Houghton and Raman, 1998). Steroid with Liebermann-Buchard test consisting of a mixture of glacial acetic acid and sulphuric acid (Shoppee, 1964**).** Terpenoids with a mixture of extract and chloroform and concentrated H2SO4 (Sofowora, 1993). Anthraquinones with Borntragers test consisting of filtrate from the mixture of the extract and benzene with 10% ammonia solution (Sofowora, 1993).

Assessment of Hypoglycemic Potentials of *Nicotiana tabacum* (Single Dose Study): Fifteen rats were randomly selected into 3 groups, 5 rats per group. The rats were fasted overnight. Fasting blood glucose levels of each group was evaluated. Group I, untreated control was given 0.5ml of 2% Acacia, while Group II and III were given the extract, orally at doses of 40mg/kg and 80 mg/kg respectively. Blood samples were collected for estimation of Blood glucose level from the tail vein at 2, 4 and 6h after giving the extract (Santosh *et al*., 2007, Emordi *et al*., 2015).

Effect of the Extract on Oral Glucose Tolerance Test (OGTT): Normal rats male and female were fasted over night and divided into five groups of five rats each. Blood samples were collected from the tail veins of the rats to estimate the fasting blood glucose levels. The control, was given 2% Acacia and group 2, 3, 4 and 5 were given 20, 40, 80mg/kg of extract and 5mg/kg of Glibenclamide respectively. Thirty minutes after, the rats in each group were administered 40% (w/v) glucose at a dose of 1ml/100g body weight orally (Ogbonnia *et al*., 2011). Blood glucose levels monitored at 30minutes, 60minutes and 120 minutes intervals and reported as the average glucose level of each group.

Statistical analysis: Analysis of Data was done using Graph Pad Prism 6. One way analysis of variance and t-test were used to compare means. Level of significance was set at $p<0.05$

RESULTS

Acute Toxicity Study: In the acute toxicity study (Table1), there was no death among the mice that received 1000-2500mg/kg body weight of the extract. One of the mice that received 5000mg/kg died within 24hrs. The mice that received 10000-20000mg/kg body weight of the extract died within 24hrs.The LD_{50} of the drug was calculated to be 5821mg/kg

The phytochemical screening of the leaf extract of *Nicotiana tabacum* revealed the presence of flavonoids, alkaloids, phenol, cardiac glycosides, and terpenoids. (Table 2)

Table1: Oral acute toxicity of the leaf extract of *Nicotiana tabacum*

Group	Dose(mg/kg)	Log dose	No of Deaths	% Death	Probit Value
I	1000	3.0	0	0	0
II	2500	3.4	0	0	0
III	5000	3.7	1	20	4.16
IV	10000	4.0	5	100	8.09
V	15000	4.18	5	100	8.09
VI	20000	4.30	5	100	8.08

n =5, Control received 0.4ml of 2% acacia

Table 3 shows the hypoglycaemic effects of a single oral administration of two doses 40mg/kg and 80mg/kg body weight of the leaf extract of *Nicotiana tabacum* in normal healthy rats. The 40mg/kg dose showed a significant reduction ($p<0.05$) in blood glucose level at 2h compared to control. The reduction in blood glucose level at the dose of 40mg/kg body weight at 2h, 4h, and 6h was 27.35%, -14.47% and 23.23% respectively. The 80mg/kg dose also showed a significant reduction ($p<0.05$) in blood glucose level at 2h and 6h compared to control. The 80mg/kg body weight of the extract showed a maximum reduction of 75.40% in blood glucose level after 6hr of oral administration. The reduction in blood glucose level at the dose of 80mg/kg body weight at 2 and 4h was 28.37% and 2.09% respectively.

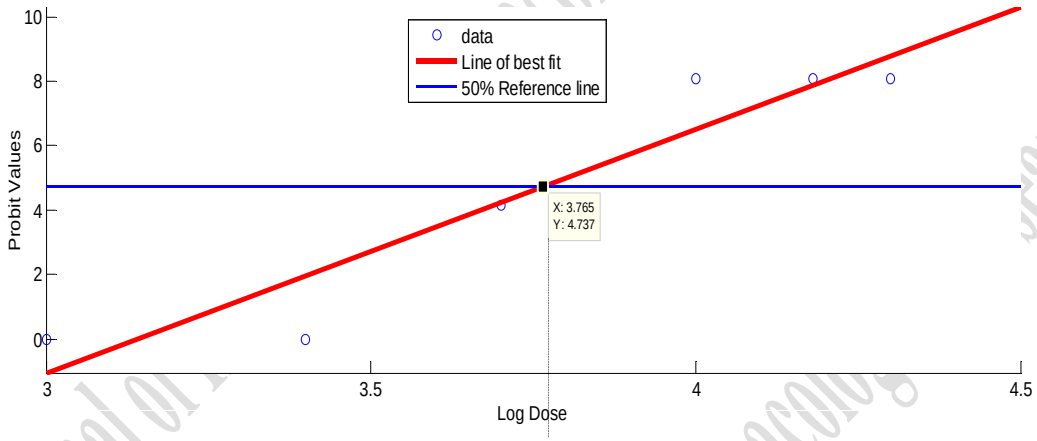

Figure 1: LD_{50} of *Nicotiana tabacum*

Table 2: Qualitative analysis of the phytochemical constituents of the leaf extract of *Nicotiana tabacum*

Compounds	Presence
Flavonoids	+++
Alkaloids	++
Phenol	+++
Tannin	-
Saponin	-
Cardiac glycosides	+++
Anthraquinone	-
Steroid	-
Terpenoids	+

+++appreciable amount; ++ moderate amount; + trace amount; -not present

Table 3: Assessment of hypoglycaemic potentials of *Nicotiana tabacum*

	Blood Glucose Levels (mg/dl)			
	Pre-treatment	Post-treatment		
Group	0 (FBG)	2hrs	4hrs	6hrs
I	62.2 ± 2.54	63.8 ± 2.40	53.8 ± 2.48	66.3 ± 7.00
II	64.9 ± 2.62	50.1 ± 3.4*	62.9 ± 3.96	53.8 ± 4.76
III	54.5 ± 2.88	49.7 ± 2.46*	52.7 ± 3.81	37.8 ± 2.71*

Mean ± SEM; n=5; *p<0.05 vs. control group; Group I: control received 0.5 mL of 2% acacia; Group II: 40 mg/kg of extract; Group III: 80 mg/kg of extract.

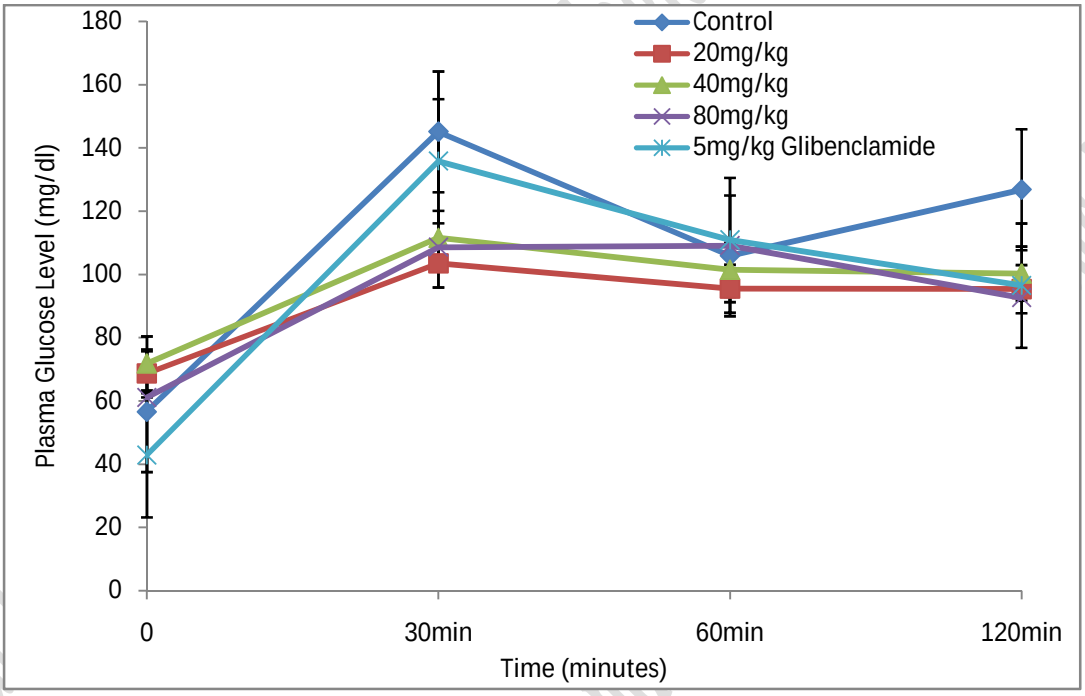

Fig.2: Oral glucose tolerance test

Figure 2 shows the summary of the Oral Glucose tolerance Test (OGTT). Following oral glucose load, there was a marked increase in blood glucose levels of the rats in the control group and those treated with 5mg/kg glibenclamide. The increase in blood glucose levels reached a peak at 30mins of glucose load. Decrease in blood glucose levels occurred after 30mins for both groups. There was an initial rise in the blood glucose level in the control group after 60mins which was sustained in the next 1hr. While the blood glucose levels of the rats treated with the reference drug, glibenclimide, showed a consistent decrease after 120mins. However, the doses of 20mg/kg, 40mg/kg and 80mg/kg body weight of the extract showed a significant reduction ($p<0.05$) in blood glucose level at 30mins with a decrease of 40.19%, 30.02% and 33.61% respectively. The 80mg/kg body weight of the extract also showed a significant reduction ($p<0.05$) in blood glucose level at 120mins with a reduction of 36.88%.

DISCUSSION:

The results of this study showed that the median lethal dose (LD_{50}) of the leaf extract of *N. tabacum* was determined to be 5.82g/kg body weight translating to 407g dose for human adult. According to Loomis and Hayes (1996), the extract can be classified as being practically non-toxic since this value is much higher than the Organization for Economic Cooperation and Development (OECD) toxicity index of 2 g/kg (Walum 1998, OECD 2001). Therefore, the extract may be considered to be safe for consumption.

Diabetes mellitus is a disease of an endocrine pancreas caused by impaired metabolism of glucose, protein and lipids predisposing to hyperglycemia (Pareek *et al*., 2009). Hyperglycemia is the hallmark of diabetes and glycemic control remains the main goal of treatment in an attempt to prevent chronic complications (Miccoli *et al*., 2011)
This study revealed that the 40mg/kg body weight of the extract showed a significant reduction ($p<0.05$) of 27.35% in blood glucose level at 2h compared to control. The 80mg/kg body weight of the extract had maximum reduction of 75.40% in blood glucose level after 6hours of oral administration. The extract exerted hypoglycaemic activity by decreasing the blood glucose level significantly. Good glycemic control reduces risk of microvascular and neurological complications of diabetes (Skyler, 2004)

The result of Oral Glucose Tolerance Test (OGTT) showed that the 20mg/kg, 40mg/kg and 80mg/kg body weight of the extract showed a significant reduction ($p<0.05$) in blood glucose level at 30mins with a decrease of 40.19%, 30.02% and 33.61% respectively. The 80mg/kg body weight of the extract also showed a significant reduction ($p<0.05$) in blood glucose level at 120mins with a reduction of 36.88% compared with control.

Insulin release in response to a glucose load occurs in two phases in humans and in rodents. The early phase peaks within the first 15-30 min and is responsible for limiting the initial rise in glucose upon meal ingestion. The late phase of insulin secretion occurs later than 30 min after a meal, and may persist for several hours. This delayed burst of insulin secretion is responsible for returning glucose to baseline fasting levels (Emordi *et al.,* 2015).

From this study, the marked reduction in plasma glucose concentration may be as a result of increased release of insulin from beta cells. This may also account for the hypoglycaemic activity of the extract. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents (Wadood *et al.,* 2013). Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Saxena *et al.,* 2013). Phytochemical analysis conducted on the leaf extract of *Nicotiana tabacum* revealed the presence of flavonoids, alkaloids, phenol, cardiac glycosides and terpenoids. Flavonoids, especially quercetin have been reported to possess antidiabetic activity. (Vessal *et al.,* 2003) reported that quercetin brings about the regeneration of pancreatic islets and probably increases insulin release in streptozotocin-induced diabetic rats.

The potential therapeutic use of polyhydroxylated alkaloids in the treatment of type-2 diabetes due to their ability to inhibit maltase-glucoamylase has been reported (Shang *et al*., 2013). Triterpenoids isolated from bitter melon has shown antidiabetic activity (Tan *et al*., 2008). It is therefore possible that the phytochemicals present in the leaf extract of *Nicotiana tabacum* may be responsible for the observed hypoglycaemic activity in this study.

CONCLUSION

The LD50 value 5.82g/kg obtained was a clear indication that *Nicotiana tabacum* is safe for use. The study showed that the leaf extract of *Nicotiana tabacum* has hypoglycemic activity which may be as a result of increased release of insulin from beta cells. The hypoglycemic activity of the extract may also be accounted for by the presence of several bioactive compounds like flavonoids, alkaloids, phenols and terpenoids.

ACKNOWLEDGEMENT:

The authors wish to thank Mr. M.E Idemudia of Idumu-une Quarters,Uromi, Edo State, Nigeria, for his assistance in plant collection.

REFERENCES

Alberti, K.G.M.and Zimmet, P.Z (1998). Definition, diagnosis and classification of Diabetes mellitus and its complications. Part1: Diagnosis and classification of diabetes mellitus. Provisional report of WHO consultation. *Diabet Med*; 15: 539-553.

Bakht, J. A. and Shafi, M. (2012). Antimicrobial activity of *Nicotiana tabacum* using different solvents extracts. *Pak J Bot*; 44:459-463.

Baron, J.A. (1986). Cigarette smoking and Parkinson's disease. *Neurology*; 36:1490-1496.

Burger, C., Fischer, D.R., Cordenuzzi, D.A., Batschauer de Borba, A.P., Filho, V.C. and Soares dos Santos, A.R. (2005). Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* in mice. *J Pharmaceut Sci*; 8: 370-373.

Emordi, J.E, Agbaje, E.O., Oreagba, I.A. and Iribhogbe, I.O. (2015). Preliminary phytochemical screening and evaluation of hypoglycemic properties of the root extract of *Uveria chamae*. *Bangladesh J Pharmacol*; 10: 326-331.

Farnsworth, R.N. (1966). Review on biological and phytochemical screening of plants. *J Pharm Sci*; 55: 225-276.

Harborne, J.B. (1998). Phytochemical methods: A guide to modern techniques of plant analysis. 3rd ed. London, Chapman and Hall, p. 235.

Houghton, P.J. and Raman, A. (1998). Laboratory handbook for fractionation of natural extracts. London, Chapman and Hall, p.199.

King, H., Aubert, R.E. and Herman, W.H. (1998). Global burden of Diabetes, 1995- 2025 prevalence, numerical estimates and projections. *Diabetes Care*; 21(9):1414-1431.

Ksir, C. and Benson, D.M. (1983). Enhanced behavioral response to nicotine in an animal model of Alzheimer's disease. *Psychopharmacology*; 81:272-273.

Loomis, T.A. and Hayes, A.W. (1996). Loomis's essentials of toxicology. 4th ed. California, Academic press 208-245.

Mbaka, G.O., Ogbonnia, S.O. and Banjo, A.E. (2012). Activity of *Raphia hookeri* root extract on blood glucose, lipid profile and glycosylated haemoglobin on alloxan-induced diabetic rats. *J Morphol Sci.* 29: 214-222.

Mbaka, G.O., Ogbonnia, S.O., Oyeniran, K. J. and Awopetu, P. I. (2012). Effect of *Raphia hookeri* Seed Extract on Blood Glucose, Glycosylated Haemoglobin and Lipid Profile of Alloxan Induced Diabetic Rats. *Br J Med Med Res*; 2(4): 621-635

Miccoli, R., Penno, G. and Del Prato, S. (2011).Multidrug Treatment of Type 2 Diabetes: A challenge for compliance. *Diabetes Care*; 34 (Suppl. 2).

National Research Council (NRC) (2011). Guide for the care and use of laboratory animals. 8th ed. The National Academies Press. Washington, DC.

OECD (2001). OECD Guidelines for the Testing of Chemicals, No 420. Acute oral toxicity- Fixed Dose Procedure.Paris, France: Organization for Economic Cooperation and Development..

Ogbonnia, S.O., Odimegwu, J. I. and Enwuru, V.N. (2008). Evaluation of hypoglycaemic and hypolipidaemic effects of aqueous ethanolic extracts of *Treculia africana* Decne and *Bryophyllum pinnatum* Lam. And their mixture on streptozotocin (STZ)-induced diabetic rats. *Afr. J. Biotech*. 7(15), 2935-2939.

Ogbonnia, S.O., Mbaka, G.O., Anyika, E.N., Ladiju, O., Igbokwe, H.N., Emordi, J.E. and Nwakakwa. N. (2011). Evaluation of anti-diabetics and cardiovascular effects of *Parinari curatellifolia* seed extract and *Anthoclista vogelli* root extract individually and combined on postprandial and alloxan-induced diabetic albino rats. *Br J Med Medical Res*; 1: 146-162.

Pareek, H., Sharma, S., Khajja, B.S., Jain, K. and Jain, G.C. (2009). Evaluation of hypoglycaemic and anti-hyperglycaemic potential of *Tridax procumbens* (Linn). *BMC Comp. Alter. Med*; 9 (48): 1-9.

Santosh, K.S., Achyut, N.K., Prashant, K.R. and Geeta, W. (2007). Assessment of glycemic potential of *Musa paradisiacal* stem juice. *Indian J Clin Biochem*; 22: 48-52

Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A. (2013). Phytochemistry of Medicinal Plants. J *Pharm Phytochem*. 1(6): 168-182

Sela-Buurlage, B. M., Ponstein, S.A., Bres-Vloemans, A.S., Melchers, S.L., van den Elzen, J. M. P. and Cornelissen J.C. B. (1993). Only Specific Tobacco (Nicotiana tabacum) Chitinases and 8-1,3-Clucanases Exhibit antifungal activity. *Plant Physiol*; 101: *857-863*.

Shah, A.M.A., Garg, S.K. and Garg, K.M. (1997). Subacute toxicity studies on pendimethalin in rats. *Indian J Pharmacol*; 29: 322-324.

Shang, Q., Xiang, J., Zhang, H., Li, Q. and Tang, Y. (2013). The effect of polyhydroxylated alkaloids on maltase-glucoamylase. *PLoS ONE*; 8: 1-5.

Shaw, J.E, Sicree, R.A. and Zimmet, P.Z (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabet Res Clin Pract*; 87: 4–14.

Shoppee, C.W. (1964). Chemistry of Steroids. 2nd edition, Butterworths, London, p.326

Skyler, S. J. (2004). Effects of Glycemic Control on Diabetes Complications and on the Prevention of Diabetes. *Clin Diabet*; 22(4):162-166.

Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. Ibadan, Nigeria, Spectrum Books limited. pp. 191-289.

Suneetha, B., Sujatha, D. and Prasad, K. (2010). Antidiabetic and antioxidant activities of stem juice of *Musa paradisiaca* on alloxan induced diabetic rats. *Int J Adv Pharmaceut Sci*; 1: 167-176.

Tan, M., Ye, J., Turner, N., Hohnen-Behrens, C., Ke, C., Tang, C., Chen, T., Weiss, H., Gesing, E., Rowland, A., James, D. and Ye, Y. (2008). Antidiabetic Activities of Triterpenoids Isolated from Bitter Melon Associated with Activation of the AMPK Pathway. *Chem Bio*; 15: 263–273

Trease, G.E. and Evans, W.C. (1996). Pharmacognosy. 4th ed. USA, W.B.Saunders, pp. 243-83.

Turner, A.P.F., Chen, B. and Piletsky, S.A. (1999). In vitro Diagnostics in diabetes meeting the challenge. *Clin. Chem*; 45:9: 1596-1601.

Tzourio, C., Rocca, W.A., Breteler, M.M., Baldereschi, M., Dartigues, J. F., Lopez-Pousa, S., Manubens-Bertran, J.M. and Alperovitch, A. (1997). Smoking and Parkinson's disease An age-dependent risk effect? *Neurology*; 49: 1267-1272

Vessal, M., Hemmati, M. and Vasei, M. (2003). Antidiabetic effects of quercetin in streptozocin induced diabetic rats. *Comp. Biochem. Physiol* C.; 135: 357-364.

Wadood, A., Ghufran, M., Jamal, S.B., Naeem, M., Khan, A. *et al*. (2013). Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. *Biochem Anal Biochem*; 2: 144.

Walum, E. (1998). Acute oral toxicity. *Environmental health perspectives*; 106 (2):498.

Whitehouse, P.J., Martino, A.M., Antuono, P.G., Lowenstein, P.R., Coyle, J.T., Price, D.L. *et al*. (1986). Nicotinic acetylcholine binding in Alzheimer's disease. *Brain Res*; 371:146-151.

Wilson, A.L., Langley, L.K., Monley, J., Bauer, T., Rottunda, S., McFalls, E. *et al* (1995). Nicotine patches in Alzheimer's disease: pilot study on learning, memory, and safety. *Pharmacol Biochem Behav*; 51:509-514.

World Health Organization (1980). Expert committee on diabetes mellitus. (Technical Report, Series 646). Geneva, WHO

World Health Organisation(1999). Department of Noncommunicable Disease Surveillance. Definition, and classification of Diabetes mellitus and its complication.

Yadav, R.N.S. and Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. *J Phytol*; 3: 10-14.

Zimmet, P. and McCarty, D. (1995). The NIDDM epidemic. Global estimates and projection: A look into the crystal ball. *IDF. Bull;* 40: 8-16.

AUTHORS CONTRIBUTIONS

Emordi, J.E, Agbaje, E.O. and Oreagba, I.A. conceived this study. The experiments were carried out by Emordi, J.E., Iribhogbe, O.I. and Ota, D.A., while Emordi, J.E. drafted the manuscript and supervised the review process. No conflict of interest is declared.