Advances in Environmental Biology, 3(1): 33-38, 2009
ISSN 1995-0756
© 2009, American-Eurasian Network for Scientific Information

This is a refereed journal and all articles are professionally screened and reviewed

ORIGINAL ARTICLE

Comparative Studies on the Hypoglycaemic, Hypoproteinaemic, Hypocholesterolaemic and Hypolipidaemic Properties of Ethanolic and Normal Saline Extracts of the Root of *Vernonia Amygdalina* in Diabetic Rats.

[1]Igbakin, A.P. and Oloyede[2], O.B.

[1]*Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.*
[2]*Department of Biochemistry, University of Ilorin, Ilorin, Nigeria.*

Igbakin, A.P. and Oloyede, O.B.; Comparative Studies on the Hypoglycaemic, Hypoproteinaemic, Hypocholesterolaemic and Hypolipidaemic Properties of Ethanolic and Normal Saline Extracts of the Root of *Vernonia Amygdalina* in Diabetic Rats., *Am.-Eurasian J. Sustain. Agric., 3(1): 33-38, 2009*

ABSTRACT

A comparative study of the hypoglycaemic, hypoproteinaemic, hypolipidaemic and hypocholesterolaemic properties of the ethanolic and normal saline extracts of the root of *vernonia amygdalina* was carried out on alloxanized diabetic rats treated for seven weeks. Results showed that the ethanolic extract of the root of *vernonia amygdalina* (EEVA) is more potent as it lowered the blood glucose by 68% while the Normal Saline extract of the root of *V. amygdalina* (NEVA) only reduce same by 24%. The lowering effects of EEVA on the serum protein, cholesterol and total lipid were also significant ($p<0.05$) when compared with rats on NEVA and diabetic untreated groups. The results clearly indicate that the hypoglycaemic, hypocholesterolaemic, hypolipidaemic and hypoproteinaemic active principles is contained in the ethanolic extract of the root of *Vernonia amygdalina* and not the normal saline extract. The various concepts associated with metabolism of sugar, proteins, and lipids in diabetic state are also discussed.

Key words: Alloxannized diabetic rats, hypocholesterolaemic, hypolipidaemic, hypoglycaemic, hypoproteinaemic and *Vernonia amygdalina*.

Introduction

Vernonia amygdalina (Compositae) is one of the edible vegetables in Nigeria and other parts of African sub-regions. It is the name of an African shrub or small trees of the aster family, called bitter leaf [6]. As a vegetable it contains antinutrient substances like alkaloids, saponnins and flavones that have medicinal properties [7,17] Studies have shown that extracts of the flowers of *Vernonia amygdalina* exhibited a pronounced antiviral effect against several of the test viruses and it has been employed in the treatment of Asthma and kidney problems in Brazil and some part of Asia [22]. Several other antinutrients isolated from the plant included vernoleptin which has spamolytic activity *in vitro* on smooth muscle of guinea pigs; eramathine which inhibits the penetration of cercariae of nematode schistosoma mansonii [3]. Other physiologically active compounds isolated from this plant include sesquiterpene lactones-vernodaline and vernomyelin, a sterol-7,24 (28)-stigmastadiene-3β-ol; toxic cardenolides and saponins. All these components have been proved to have physiological and metabolical effects on the body system.

In Nigeria where the plant is found in abundance, it performs both medicinal and nutritive functions, it is used in the preparation of soup, prevention of malaria fever, elimination of worms, treatment of stomach upset, induction of fertility in

Corresponding Author

Igbakin, A.P., Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.
Email: isowande@yahoo.com

barren women and treatment of diabetic mellitus [11]. The leaves and the bark/stem have been acclaimed to have anti-diabetic properties in different solvents. Nothing has been documented reasonably on the anti-diabetic properties of the root. This paper attempts to assess and compare the hypocholesterolaemic, hypoglycaemic, hypolipidaemic and hypoproteinaemic effect of different solvent extracts of the root of *Vernonia amygdalina*.

Materials and methods

Equipments and chemicals

Bovine serum albumin (BSA), copper(II)ulphate, potassium iodide, cholesterol colour reagent, cholesterol standard, vanillin, alloxan and bromocresol green are products of sigma chemical company Mo, USA while chloroform, ethanol, phosphoric acid, sulphuric acid, glacial acetic acid and ferric chloride were purchased from BDH chemicals Poole, England, olive oil is a product of May and Baker. All reagents are of analytical grade, spectro 21 Bausch and Lomb made in USA, laboratory table centrifuge Model SM 800B uniscope SM 8001A laboratory water bath and wrist action shaker made in USA were used for the analysis.

Animals

Post weanling healthy albino rats of the wister strain (*Rattus norvegicus*) weighing between 145 and 158g were used for the study. They were fed commercially produced diet (Bendel feed Nig. Ltd. Benin city, Nigeria) *ad libitum*. They were maintained at 25-27°C and kept under 12 hours of light and 12 hours of darkness. They were also allowed free access to drinking water in a well ventilated animal cage.

Plant samples

The roots of *Vernonia amygdalina* was obtained from various locations in Ilorin sub-towns during the month of many 2002; it was air-dried in the laboratory for 20 days at 26-29°C. the plants root was identified and authenticated by Professor M.A. Faluyi of the plant science Department of university of Ado-Ekiti, Ekiti State, Nigeria.

Preparation of root extract of Vernonia amygdalina

The dried root was finely grounded into powder after which 5g portions were separately macerated with 250cm ^{3} of 2% ethanol (v/v) and 0.9% NaCl (w/v) solutions and kept at room temperature for 24 hours to allow extraction to take place. The resulting mixtures were vigorously shaken for 6 hours by wrist action shaker for complete extraction to be achieved. The extracts were separately filtered and evaporated under reduced pressure The resulting residues were separately re-extracted with 2% (v/v) ethanol and 0.9%(w/v) NaCl solution and filtered again. The two filtrates were pull together and the weight of the remaining residues computed. The filtrate was reconstituted to a final concentration of 500 mg/ml in each case and kept frozen until ready for use.

Experimental design

The rats were divided into six groups with each group consisting of nine rats. Five of these groups were made diabetic by a single intraperitoneal injection (100mg/kg body weight) of alloxan in normal saline. Blood glucose of rats more than 180mg/dl was chosen as criteria for diabetes [13]. The grouping of the animals are as follows:

NDDW: Non diabetic rats on distilled water (control)
DEVA: Diabetic rats on 500mg/kg body weight of ethanolic extract of *Vernonia amygdalina*
DNVA: Diabetic rats on 500mg/kg body weight of normal saline extract of *Vernonia amygdalina*
DETH: Diabetic rats on 2% ethanol
DNOS: Diabetic rats on normal saline
DDSW :Diabetic rats on distilled water.

The extracts were administered orally to the animals for seven weeks. At the end of the seventh week the rats were sacrificed and their sera collected for biochemical analysis.

Biochemical analysis

After 24hours of alloxan administration to induce diabetes, plasma glucose was determined from the whole blood drawn from the tail vein using Trinder methods of 1969; plasma and tissue proteins were determined by the method of Gornall *et al* [9]. Plasma cholesterol was determined by enzymatic method of Trinder [25] and plasma lipids was extracted and estimated by the method reported by Strova and Markarova [24].

Statistical analysis

The data obtained were expressed as mean ± SEM and analysed statistically. The differences in values between pairs of diabetic (untreated) rats and diabetic rats treated with ethanolic extract of the root of *Vernonia amygdalina* (EEVA), between pairs of control rats and diabetic treated with normal saline

extract of the root of *Vernonia amygdalina* (NEVA), between rats on EEVA and NEVA were compared. The mean differences were analysed for significance by using Duncan multiple range test (DMRT) for the samples [20]. Only p values less than 0.05 were considered statistically significant.

Results and discussions

Figure 1 summarizes the plasma glucose of the animals employed in this study. Hyperglycaemia is the major symptom in diabetics and when glucose elimination occurs in the urine it becomes glucosuria [14]. All the diabetic rats in this present study were hyperglycaemic except the control. DNVA, DNOS, DETH and DDSW had their plasma glucose levels above 200mg/dl throughout the seven weeks of the experiment. These values are high and statistically significant ($p<0.05$) when compared with the control (NDDW) and DEVA. Many researchers had reported this trend of increased plasma glucose level in diabetic animals [4,19,15]. Plasma hyperglycaemia that occurred in diabetics has to do with the complete absence or relative deficiency of insulin which suppose to activate uptake of glucose from the plasma, utilization of glucose by peripheral tissues, inhibition of gluconeogenesis and many other functions that enable quick removal of glucose from the plasma [18].

The mechanism by which the ethanolic extract of the root of *vernonia amygdaliona* (EEVA) reduced plasma glucose level as manifested in this present study is yet to be elucidated. Although there are many possibilities, some of which may include the followings: enhancement of glucose utilization, possession of insulin-like properties and therefore induce transport of glucose into the peripheral tissues, ability to repair the pancreas to be responsive to glucose concentration for insulin production, may be acted upon and transferred by enzymes to an intermediate which possesses a pharmacological property such as hypoglycaemic property, it may contain biomolecules that can modify or stimulate insulin receptors, may modify the structure of glucose transport protein (GLUT 4) and it may inhibit insulin antagonist within the body

Plasma protein concentration

Increased plasma protein was observed in this present study (Figures 2). Animals in various groups such as DNVA, DETH, DNOS and DDSW had their plasma protein increased significantly ($p<0.05$) when compared with DEVA, and NDDW (control). There was no significant different ($p<0.05$) between the control group (NDDW) and DEVA. The various functions of insulin which is deficient in diabetics such as promoter of protein synthesis, enhancement of amino acids uptake by peripheral tissues, and ribosomes, decrease protein catabolism and decreased release of gluconeogenic amino acids etc resulted in the maintenance of constancy in the plasma protein concentration [27]. When insulin is not available or when there is availability of non-functional insulin, the reverse of all the processes above occurs. With this, the plasma protein level is supposed to decrease too. What actually increased plasma protein level in diabetics is not unconnected with alteration in physiological processes and complicative damage of the syndrome on the body tissues [16]. Hyperproteinaemia had been implicated to occur under the following conditions: chronic nephritis at the stage of polyuria which associate tightly with diabetes mellitus, renal tubular damage at the stage of recovery with gross diuresis as found in diabetic patients, uncomplicated liver disease such as acute hepatitis, hepatic necrosis, cirrhosis, biliary or portal together with vascular diseases and severe infections which characterized diabetic condition and severe diabetic ketosis resulted in elevated plasma protein [21,5,2].

Ethanolic extract of the root of *vernonia amygdalina* (EEVA) was able to reduce the plasma protein level to appreciable level of the control probably because of their abilities to prevent diabetic complications such as kidney and liver damage or to prevent hyperglycaemia which is the origin of all the diabetic abnormalities.

Changes in plasma total lipids

Total lipids levels in the plasma of diabetic rats in this present study is high (Figure 3). There was significant ($p<0.05$) difference in the level of total lipids of DNVA, DETH, DNOS and DDSW as compared with NDDW (control) and DEVA. High level of total lipids in diabetics (hyperlipidaemia) had earlier been reported by Adoga [1] and Godwin and Mohammed [8]. Total lipids in the serum include FFA, cholesterol, triglycerides and fractionation of lipoproteins. All these plasma lipids components are elevated in diabetic state as a result of relative absent or deficiency of insulin [12]. Insulin activates lipoproteins lipase that clears triglycerides from the plasma. It inhibits hormone sensitive lipoprotein lipase in the adipose tissues that release fatty acids into the plasma. With high concentration of lipids in the plasma lipoprotein synthesis equally occurs in the liver. All these resulted into accumulation of lipids in the plasma. With the treatment of diabetic rats with ethanolic extracts of the root of *vernonia amygdalina* (EEVA) the cells may revert to glucose as a source of energy or enhance responsiveness to insulin action thereby improving triglyceride clearance and decreasing lipolytic activities, resulting in lowered plasma total lipids and cholesterol as reported in this present study.

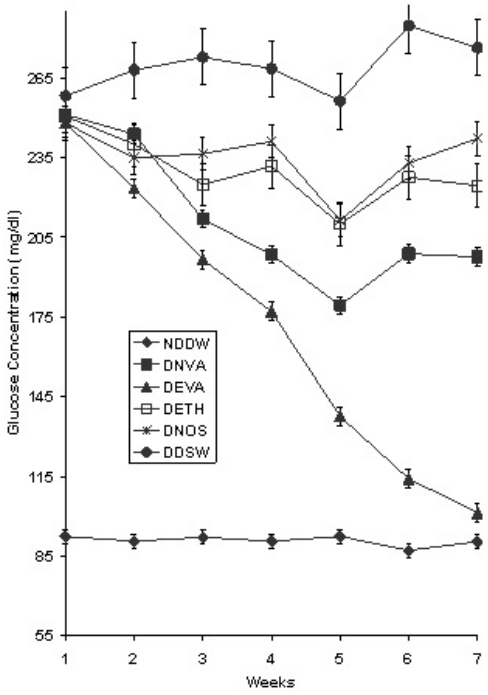

Fig. 1: Plasma glucose concentration (mg/dl) of alloxan-induced diabetic rats administered normal saline and ethanolic extracts of the root of *Vernonia amygdalina* over a period of seven weeks.

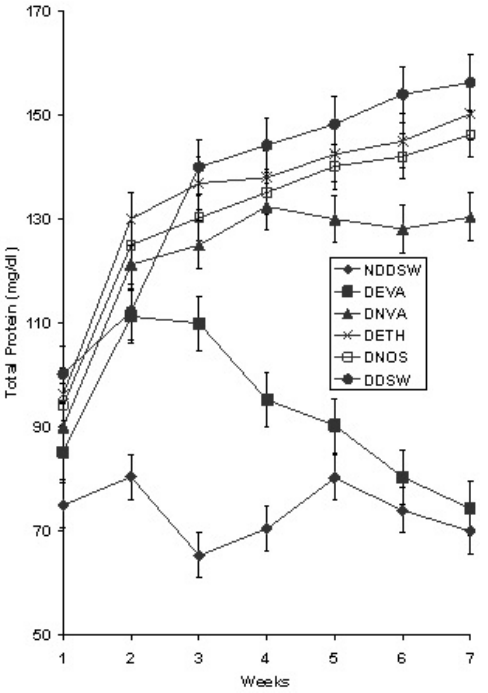

Fig. 2: Plasma protein concentration (mg/dl) of alloxan-induced diabetic rats administered normal saline and ethanolic extract of the root of *Vernonia amygdalina* over a period of seven weeks.

Cholesterols in the plasma

Cholesterol in the plasma of the animals in the DNVA, DETH, DNOS and DDSW groups increased significantly ($p < 0.05$) as compared with those in the NDDW (control) and DEVA (Figure 4). It has earlier been reported that experimentally-induced diabetes is accompanied by a concomitant increase in the serum cholesterol and lipid i.e. hypercholesterolaemia and hyperlipidaemia respectively [26,10,23]. The hypercholesterolaemia is a consequence of the body reverting to the use of lipids for energy production in the absence of glucose. This resulted to accelerated fatty acids β-oxidation that led to enormous

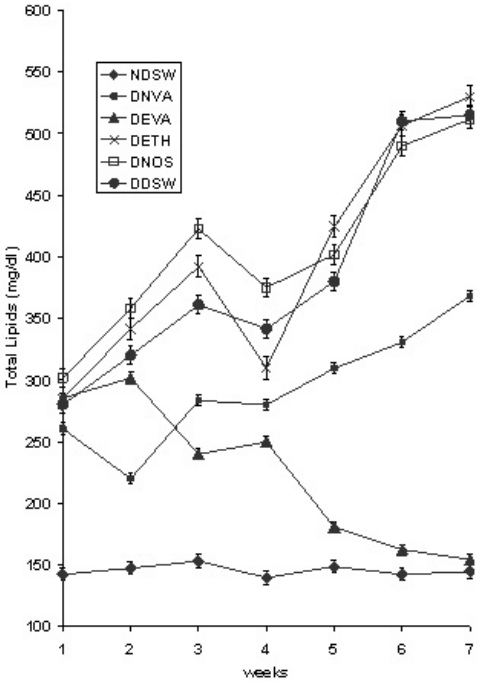

Fig. 3: Plasma total lipid concentration (mg/dl) of alloxan-induced diabetic rats administered normal saline and ethanolic extracts of the root of *Vernonia amygdalina* over a period of seven weeks.

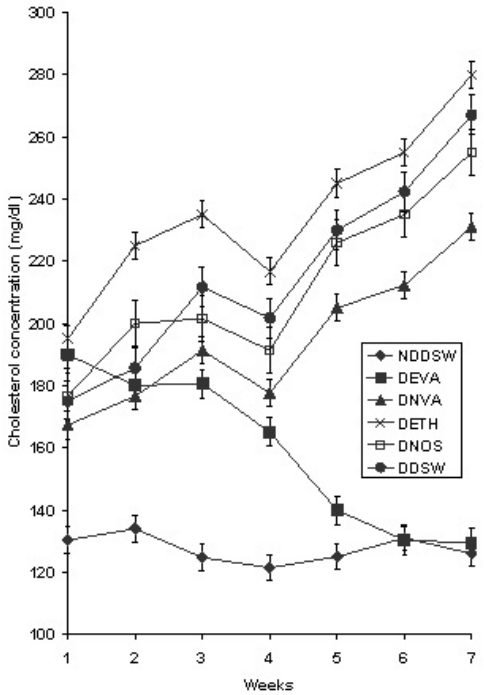

Fig. 4: Plasma cholesterol concentration (mg/dl) of alloxan-induced diabetic rats administered normal saline and ethanolic extracts of the root of *Vernonia amygdalina* over a period of seven weeks.

production of acetyl-coA, excess of which is channeled to cholesterol biosynthesis. Ability of the ethanolic extracts of the root of *vernonia amygdalina* (EEVA) to reduce the cholesterol levels in diabetes may be probably due to the fact that these plant materials contain bioactive molecules that possessed cholesterol lowering action or insulin-like action or action which can inhibit oxidation of fatty acids for energy production.

Conclusively this work has been able to show that the ethanolic extract of the root of *Vernonia amygdalina* has hypoglycaemic, hypolipidaemic, hypoproteinaemic and hypocholesterolaemic properties more than the normal saline extract of the

same plant. It equally showed that the bioactive ingredient useful in the treatment of diabetic mellitus is present in the ethanolic extract of the root of *Vernonia amygdalina* (EEVA) and not in the normal saline extract. This ethanolic extract can be further separated and purified so as to identify and determine the structure of the bioactive compound.

References

1. Adoga, G.I., 1990. Effect of garlic oil on some plasma enzymes in streptozotocin induced diabetic rats. Med. Sci. Res., 18: 528-923.
2. Andrew, H. and P. Goshin, 1999. Microalbuminuria, yet another cardiovascular risk factor? Ann. Clin. Biochem, 33: 700-703.
3. Barker, P.M., C.C. Fortess, E.G. Fortess, C. Gazzinelli, B. Gilbert, J.N.C. Borpes, J. Pellagrina, T.C.B. Tomassliv and S.K. Vichnewski, 1972. Eramathine, castinolicale cyclocostunlide and Bissabol. J. Pharmacol, 24, 853-857.
4. Ayetobi, S.O., 1995. Investigation of acclaimed hypoglycaemic and lipolipidoaemic properties of intact islets of Langarhans from the rat pancreas. Diabetes., 16, 35-39.
5. Borch-Johnson, K. and S. Kreiner, 1987. Proteinuria values as mediator of cardiovascular motality in insulin dependent diabetes mellitus. Br. Med. J., 294: 1651-1654.
6. Daziel, J.M., 1959. The useful plants of west Tropical Africa Millbank, London S.W. 1: 560-561.
7. Germaniel, K.S. and P.A. Akah, 1996. Analysis of gastrointestinal relaxing effect of the stem extract of Gongronema latifolum. Phytomedicine, 2(4): 293-296.
8. Godwin, I.A. and B.I. Mohammed, 1991. Effects of garlic oil on hypercholesterolemia in streptozotocin induced diabetic rats. Bioscience. Res. Comm., 3(2): 149-153.
9. Gornall, W.T., S.A. Watson and R.T. Jef, 1949. Estimation of total proteins by bind techniques. J. Chem., 19(6): 800-806.
10. Harris, R.A. and B.W. Grabbs, 1982. Metabolic interrelationship. In: textbook of biochemistry with clinical correlations (Delvin J. Med.). John Willey and sons. Inc. New York., 531-559.
11. kafaru, E., 1994. Immense help for natures workshop published by Elkaf. Health Services Ltd. Benin-City, 54-58.
12. Kalkhoff, R.K., K.R. Hornbook, H.B. Bwarch and D.M. Kipnis, 1966. The metabolic effects of acute insulin deficiency II changes in the hepatic glycolytic and Kreb-cycle intermediates and pyridine nucleotide. Diabetes, 15: 451-454.
13. Lacy, P.E. and M. Kostianosvky, 1963. Methods for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes, 16: 35-39.
14. Michael, W.S. and P. Daniel, Jnr., 2005. Diabetes obesity and the brain. Science, 307 (5708): 375-379.
15. Mitchell, A.L., 2005. How Obesity causes diabetes; not a tall tale. Science, 307(5708): 373-375.
16. Morrish, N.J., L.K. stevens, J.H. Fuller, H. Keen and R.J. Jarret, 1999. Incidence of microvascular diseases in diabetes mellitus: the London Conference of WHO multinational study of vascular diseases in diabetes. Diabetologia, 34: 534-589.
17. Nwafor, S.U.P.A., C.O. Akah, 2001. Potentials of plant products as anti-cancer.J. of Natural Remedies, 1: 75-88.
18. Omoruyi, F. and I. Adamson, 1998. Increased hepatic metabolism of glucose in streptozotosin induced diabetic rats fed dikanut (Iringia gabonesis) and cellulose. Biosci. Res. Comm., 6 (1): 11-16.
19. Osadebe, P.O. and U. Ajali, 2001. Antidiabetic activities of ethanolic extracts of dried Aloe barbadenis (Lin) plant. Nig. I. Biochem. And Mol. Biology, 16(3): 79-81.
20. Parker, R.E., 1973. Introductory statistics for biology, studies in biology No. 43, Edward Arnold, London, 120-124.
21. Rothchild, M.A., M. Oratz and Schreiberg, 1972. N. Enyl. J. Med., 286(748): 816.
22. Rwangabo, A.T., 1986. Antiviral effect of extract of amydalina. Planta Medica., 1-6: 547-549.
23. Smith, S.L., R.L. Hull, J.R. Lehman, R.J. Lefkoroitz, P. Handles and A. White, 1983. Principles of Biochemistry, 7th ed. Med. Granhill book company, London, 565-569.
24. Strova, E.A. and Markarova, 1989. Determination of serum lipids, laboratory manual in biochemistry. Mur Publishers, Moscow, 148-150.
25. Trinder, P., 1969. Determination of glucose in the blood using glucose oxidase with alternative oxygen acceptor. Ann. Clin. Biochem., 6, 24-25.
26. West, E.E., W.R. Todd, H.S. Mason and Van-J.T. Bruggen, 1966. Biochemistry, 4th ed., the Macmillian Company, Coller Macmillian LTD. London, 1017-1118.
27. William, A.O., 1999. Review of Medical Pharmacology, 20th ed. McGraw-Hill, New York, pp: 870.