Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol. 4, Suppl 1, 2011 ISSN - 0974-2441

Research Article

SUGAR-LOWERING EFFECTS OF BITTER LEAF (*VERNONIA AMYGDALINA*) IN EXPERIMENTAL BROILER FINISHER CHICKENS

***O. J. OWEN[1], A. O. AMAKIRI[1] AND T. A. KARIBI-BOTOYE[2]**

[1]Department of Animal Science, Rivers State University of Science and Technology, P.M.B. 5080, Port Harcourt- Nigeria.
[2]Department of Chemical Pathology, University of Port Harcourt Teaching Hospital,Port Harcourt- Nigeria.
Email: congomfx@yahoo.com

ABSTRACT

The anti-diabetic efficacy of bitter leaf (*Vernonia amygdalina*) leaf meal (VALM) was evaluated using broiler finishers'. Using 0%, 5%, 10% and 15% VALM representing diets A,B,C,D respectively to replace groundnut cake (GNC) in broiler finishers' feed, a total of 144 Marshal brooded broilers weighing 500 – 610g were used in a study that lasted for 28 days. The birds were divided into 4 groups of 36 birds each. Each treatment group was further subdivided into 3 replicates of 12 birds in a Completely Randomised Design (CRD). At the expiration of the experiment, 3 birds per treatment were randomly selected and bled for blood samples. The biochemical indices determined includes Glucose, Urea, Creatinine, Total protein and Globulin. The results indicated that the use of V. amygdalina did not significantly ($P>0.05$) affect the serum urea and creatinine of broiler birds. However, there was a significant ($P<0.05$) decrease in blood glucose of the chicken as the level of inclusion of VALM increases. Also significant ($P<0.05$) differences were observed in total protein and globulin of the birds. The percentage reduction of glucose were 14.30%, 22.90% and 28.60% for treatments B, C and D respectively. These results clearly indicate that the administration of *V. amygdalina* at varying levels produced hypoglycaemic effects. *V. amaygdalina* also did not seem to have adverse effect on the liver and kidney, since the serum urea and creatinine levels were not significantly altered. It could be inferred that *V. amaygdalina* besides exhibiting hypoglycaemic activity is also safe for consumption as food or medicine, since there were no indication of toxicity judging from the values of the biomolecules evaluated.

Keywords: Anti-diabetic, Biomolecules, Broilers, Hypoglycaemic, Toxicity, *V. amygdalina.*

INTRODUCTION

Diabetes mellitus has been defined by the world health organization [WHO], on the basis of laboratory findings as a fasting venous plasma glucose concentration greater than 7.8 mmol/l (140mg/dl) or greater than 11.1 mmol/l (200mg/dl) two hours after a carbohydrate meal or two hours after an oral ingestion of the equivalent of 75g glucose, even if the fasting concentration is normal (Nwanjo and Nwokoro, 2004). It is a metabolic disease characterized by hyperglycaemia and glycosuria due to absolute or relative lack of insulin (Aguwa, 1996).

Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and are still widely used and have considerable importance in International trade (Ahmad *et al.,* 2006). In certain African countries for instance, up to 90% of the population still relies exclusively on plants as a source of medicines (Hostettmann *et al.,* 2000). As a consequence, the world health organization (WHO) had in one of its charters in Geneva recommended further investigation into this area, particularly, as it concerns chronic and debilitating disease such as diabetes mellitus (WHO, 1980).

A complex disease like diabetes mellitus, where little is talked about in aspects of prevention and curation, but rather management, there is an increased focus on plants in the search for appropriate hypoglycaemic / antihyperglycaemic agents (*Ebong et al*, 2008). Firstly, because of leads provided by traditional medicine to natural products that may be better treatments than currently used conventional drugs (Rates, 2001). Secondly the plants by secondary metabolic means contain a variety of herbal and non-herbal ingredients that are thought to act on a variety of targets by various modes and mechanisms (Tiwari and Rao, 2002) given the multi-factorial pathogenicity of the disorders.

Vernonia amygdalina Del (Compositae) popularly known as bitter leaf is a shrub of 2 – 5m tall (Ojiako and Nwajo, 2006). It is popularly called bitter leaf because of its abundant bitter principles. It is cultivated in Nigeria mainly for its nutritional value (Igile *et al*., 1995a, Owen *et al*, 2009). The plant (especially the leaf) has been found useful in the ethnotheraphy of diabetes (Akah and Okafor, 1992; Uhegbu and Ogbuehi, 2004; Nwajo, 2005), asthma, schistosormiasis, malaria (Masaba, 2000), measles, diarrhea, tuberculosis, abdominal pain and fevers, cough (Akinpelu, 1999). Phytochemicals contained in *V. amygdalina* include saponins, sesquiterpenes, lactones and flavonoids, steroid glucosides such as Vernoniosides A_1, A_2, A_3, A_4, B_1, B_2, B_3, D and E have been isolated from the plant (Ohigashi, 1994; Aregheore *et al*., 1997; Igile *et al*., 1995b). But it is yet to be ascertained which of these are responsible for some observed biological effects of the plant (Ekpo *et al*, 2007). However, there is dearth of scientific data to support the folkloric use of this plant in the treatment of diabetes mellitus disease in Nigeria herbal homes. Therefore, the present work was designed to provide scientific proof of the use of *V. amygdalina* in the treatment of diabetes mellitus and we hope that the results of this short-term investigation will have important implications for the management of diabetes.

MATERIALS AND METHODS

The fresh leaves of *V. amygdalina* were collected in June, 2009 from a garden in Port Harcourt, South-South of Nigeria. The harvested leaves were spread and air-dried for 60 days and sun-dried for one additional day to ensure perfected drying, milled and packed into jute bags. Using 0%, 5%, 10% and 15% VALM to replace groundnut cake in broiler finishers' feed, a total of 144 Marshal brooded broilers weighing 500 – 610g were used in a study that lasted for 28 days in the Teaching and Research Farm of the Rivers State University of Science and Technology, Port Harcourt.

The birds were housed in a deep litter with wood shavings as litter material. Before the arrival of the birds, the pens were cleaned, washed and disinfected. The birds were divided into 4 groups of 36 birds each. Each treatment group was further subdivided into 3 replicates of 12 birds in a Completely Randomised Design (CRD). Feed and water were offered *ad libitum*. At the expiration of the experiment, 3 birds per treatment were randomly selected and bled by severing the jugular vein.

The blood samples were collected into bottles without anticoagulant and taken to the chemical pathology laboratory of the University of Port Harcourt Teaching Hospital (UPTH) for biochemical analysis. The biochemical indices determined includes Glucose, Urea, Creatinine, Total protein and Globulin. All the data collected were subjected to Analysis of Variance (ANOVA) according to Steel and Torrie (1980) and means where appropriate were partitioned using Duncans New Multiple Range Test (DNMRT) as outlined by Obi (1990).

Table 1: The effects of graded levels of *V. amygdalina* on biochemical parameters of broiler finishers

Parameters	Treatments			
	A (0% VALM)	B (5% VALM)	C (10% VALM)	D (15% VALM)
Glucose mmol/l ± SEM	$3.50^{a} \pm 0.20$	$3.00^{a} \pm 0.15$	$2.70^{b} \pm 0.19$	$2.50^{b} \pm 0.20$
Glucose reduction %	0	14.30	22.90	28.60
Urea mmol/l	3.00	3.00	3.10	3.10
Creatinine mmol/l	64.20	64.00	63.50	63.00
Total protein g/l ± SEM	$57.00^{a} \pm 0.45$	$40.00^{b} \pm 0.30$	$40.00^{b} \pm 0.15$	$40.00^{b} \pm 0.18$
Globulin g/l ± SEM	$10.00^{b} \pm 0.15$	$14.00^{a} \pm 0.20$	$14.00^{a} \pm 0.18$	$14.00^{a} \pm 0.21$

Within rows, means ± SEM with different superscript differs significantly at p<0.05. SEM: Standard Error of Means

RESULTS

Table 1 depicts the effects of *Vernonia amydalina* (VA) on the biochemical indices of broiler finisher chickens. The use of V. amygdalina did not significantly (P>0.05) affect the serum urea and creatinine of broiler birds. However, there was a significant (P<0.05) decrease in blood glucose of the chicken as the level of inclusion of VA increases. Also significant (P<0.05) differences were observed in total protein and globulin of the birds. There was no significant (P<0.5) difference in the urea levels between the treated and the control groups.

DISCUSSION

The data from the present investigation showed that *V. amaygdalina*, a plant commonly used for dietary and medicinal purposes, caused an overall glucose lowering effects on the treated birds when compared to the control group. The percentage reduction was 14.30%, 22.90% and 28.60% for treatments B, C and D having 5%, 10% and 15% VALM inclusion rates respectively. These results clearly indicate that the administration of *V. amygdalina* at varying levels produced hypoglycaemic effects. There are many bioactive constitutes present in the leaves and hence, at present, it is not certain, which of them is/are responsible for the observed effect. However, certain flavonoids in *V. amaygdalina* may confer hypoglycaemic property on the leaf extract of this plant (Ezekwe and Obiodoa, 2001).

According to the report of Igile *et al.*, (1994), the leaves of *V. amaygdalina* contains biflavonoids such as luteolin, luteolin 7 – 0 – B – glucoside and luteolin 7 – 0 – B – glucuronoside. Besides several stigmastine type saponins such as vernoniosides A_1, A_2, A_3, D_3 and C have been isolated from the leaves of *V. amaygdalina* (Jisaka *et al.*, 1992). In addition, *V. amygdalina* leaves had been reported to contain a bioactive sesquiterpene lactones such as vernolide and vernodalol (Erasto *et al* 2006). Thus, it is probable that the hypoglycaemic activity of *V. amaygdalina* as reported in this study, may be a function of its rich flavonoid content.

The study of Adewole *et al* (2007) showed that flavonoid such as quercetin improves hyperglycaemic and islet morphology in STZ – induced diabetic rats. Besides, Adewole and Caxton – Martins (2006) reported the beneficial effect of aqueous leaf extract of *Annona muricata* Linn on blood glucose leaves of STZ-induced diabetic rats. They concluded that plant bioflavonoids and coumarins may play roles in the establishment of normoglycaemia in diabetic rats. Similar inference had been drawn by (Ojewole, 2006; Akinola *et al*., 2009).

The hypoglycaemic effect at all levels of *V. amaygdalina* inclusion as observed in this work is therefore not surprising. It confirms the effectiveness of the leaves of this plant in the ethnotherapy of diabetes mellitus (Akah and Okafor, 1992). Although not fully understood, several reports have attempted insights into the hypoglycaemic mechanisms of plant extracts. There is a report that *Azadirachta indica* does not modulate insuline effect on glycogen metabolism (Chattopadhyay, 1993), rather it blocks the inhibitory effect of serotonin on insulin secretion/release in pancreas of rats mediated by glucose eventually glucose control.

Sonia and Srinivasan (1999) in their report presupposed increased peripheral glucose uptake by an inhibition on the action of insulin by inhibiting glycogenesis. Atangwho *et al*., (2007) had suggested in their earlier report on *Vernonia amygdalina* that two possible mechanisms exist: one targeting insulin production from the islet cells and the second on peripheral carbohydrate mechanism. Mechanism involving insulin productions are usually more potent (Ebong *et al*, 2008). It is not surprising therefore that *V. amaygdalina* tends to be a potent hypoglycaemic agent. Moreso, that other plants of the same family-compositae, such as *Chrysanthemum coronarium* has shown potent hypoglycaemic and anti-hyperglycaemic activity (Kin *et al*., 2006).

Serum total protein, globulin and urea; markers of liver synthetic ability were assessed after treatment for 28 days. Blood urea nitrogen (BUN) test measures the amount of nitrogen in the blood that comes from the waste product urea. Urea is made when protein is broken down in the body. It is produced in the liver and passed out of the body in urine. A primary consideration in the assessment of the efficacy of a potential therapeutic agent for hepatic injury (damage) is its effect on liver.

V. amygdalina also did not seem to have adverse effect on the kidney, since the creatinine levels were not significantly altered. This corroborates the data of Ekpo *et al*., (2007) where it was noted that administering the extract of *V. amygdalina* on rats did not significantly alter the creatinine levels. Creatinine is derived from muscles and released into the blood. It is removed from the body by the kidneys.

When creatinine level is elevated, a decrease in kidney function is suggested. It could be inferred that *V. amygdalina* besides exhibiting hypoglycaemic activity is also safe for consumption as food or medicine, since there were no indication of toxicity judging from the values of the biomolecules evaluated.

Therefore, in view of the central role of diet in diabetic management (Mann, 1980), several attempt have been made to suggest acceptable dietary regimes for diabetics. These recommendations, though beneficial to diabetic in general, are based on foreign diets. Thus, they did not take cognizance of the specific socio-economic and cultural attributes of African societies especially with respect to their eating habits (Taiwo *et al,* 2009).

Thus, dietary treatment of diabetes in Africa is still a major challenge because strict adherence to various recommended dietary regimes is often difficult (Naidu, 1992). Such problems may not arise with *V. amygdalina* because it is widely consumed as bitter leaf soup.

Their use in diabetic management agrees with Nigerian socio-cultural attributes. If the results of this work are confirmed in other animal and human experimental studies, the use of this plant in the treatment of diabetes can be justified considering the central role of diet in diabetic management. In order to formulate appropriate dietary regime for African diabetics, more studies are needed to reveal the glycaemic effects and composition of African local herbs and foodstuffs. Any therapeutic advice given to African diabetics should reflect their traditional eating habit.

REFERENCE

1. Adewole, S.O. and Caxton-Martins, E.A. (2006). Morphological and hypoglycaemic effects of *Annona muricata* Linn (Annonaceae) leaf aqueous extract on pancreatic B cells of streptozotocin – treated diabetic rats. *Afr. J. Biomed. Res*. 9: 173 -187.
2. Adewole, S.O.; Ojewole, J.A. and Caxton-Martins, E.A. (2007). Protective effects of quercetin on the morphology of pancreatic B cells of stretozotocine treated diabetic rats. *Afri. J. Traditional Compl. Alternative Med.* 4:64 – 74.

3. Aguwa, C.N. (1996). Diabetes mellitus. In: Therapeutic basis of climate pharmacy in the tropics. Optimal publishers, Enugu, Nigeria. pp. 1 – 453.
4. Ahmad, L.; F. Agil and M. Owais (2006). Modern phytomedicine. Turning Medicine Plants into Drugs. West-Susex England: John Wiley and Sons, pp: 2 – 24
5. Akah, P.A. and Okafor, C.I. (1992). Blood sugar lowering effect of *V. amaygdalina* Del. In experimental rabbit model *Phytother. Res*., 6: 171 – 173.
6. Akimola, O.S.; Akimola, O.B., and Caxton-Martins, E.A. (2009). *Vernonia amygdalina* regulates hepatic enzymes and improves liver micromatomy in experimental diabetes mellitus. *Pharmacologyonline*, 2: 1231 – 1242.
7. Akinpelu, D.A. (1999). Antimicrobial activity of *Vernonia amygdalina* leaves. *Fitoterapia J. Study Med. Plants.* 70: 432 – 435.
8. Areghore, E.M., Makkar,H.P.S and Becker,K (1997).Chemical composition and tannins in leaves of some browse plants from Delta (Central Nigeria) eaten by ruminants. Proc. Soc. Nutr. Physiol., 5:11-15
9. Atangwho, I.J.; Egbon, P.E.; Eteng, M.U. Eyong, E.U. and Obi, A.U. (2007). Effect of *V. amaygdalina* Del. Leaf on kidney function of diabetic rats. *Int. J. Pharm.,* 3 (2): 142 – 148.
10. Chattopadhyay, R.R. (1993). Effect of *A. indica* on leaf extract on hepatic glycogen in rats. *Ind. J. Pharm*, 25: 174 – 175.
11. Ebong, P.E; I.J. Atangwho; E.U. Eyong and G.E. Egbung (2008). The anti-diabetic efficacy of combined extracts from two continental plants: *Azadirachta indica (Neem) and Vernonia amygdalina* (bitter leaf). *American Journal of Biochemical and Biotechnology,* 4(3): 239 – 244.
12. Ekpo, A.; Eseyin, O.A.; Ikpeme, A.O. and Eddio, E.J. (2007). Studies on some biochemical effects of *V. amaygdalina* in rats. *Asian Journal of Biochemistry* 2 (3): 193 – 197.
13. Erasto, P.; Grierson, D.S. and Afolanyan, A.J. (2006). Bioactive sequiterpene lactones from the leaves of *Vernonia amaygdalina. J. Ethnophermacol.* 106: 117 – 120.
14. Ezekwe, C.I. and O. Obidoa (2001). Biochemical effect of *V. amaygdalina* on rat liver microsomes. *Nig. J. Biochem. Mol. Biol*, 16. 1745 – 1788.
15. Hottettmann, K. A. Marston, K. Ndjoko and J. Wolfender (2000). The potential of African plants as a source of Drug. *Curr. Org. Chem*., 4: 973 -1010.
16. Igile, G.O.; Oleszek, S.B.; Burda S.; Jurzysta,M.(1995b). Nutritional assessment of *Vernonia amygdalina* leaves in growing mice *Journal of Agric. Food Chem.,* 43:2162-2166
17. Igile, G.O.; Oleszek, S.B.; Jurzysta, M.; Aquino, R.; Tommas, N. and Pizza, C. (1995a). Vernoniosides D and E. Two novel saponins from *Vernonia amygdalina. Del. J. Nat. Prod.* 58: 1438 – 1443.
18. Igile, G.O.; Olezek, W.; Juryzysta, M. (1994). Flavonoids from *V. amaygdalina* and their antioxidant activities. *J. Agric. Food Chem* 42: 2445 – 2448.
19. Jisaka, M.; Ohigashi, H., Takagaki, T. (1992). Bitter steroid glucosides, vernonisides A_1, A_2, A_3 and related B*1*, from a possible medicinal plant *V. amaygdalina* used by wild Chimpanzees. *Tetrahedron*. 48: 625 – 632.
20. Kim, J.S.; Ju, J.B.; Choi, C.W. and Kin, S.C. (2006). Hypoglycaemic and antihyperglycaemic effect of four Korean medicinal plants in alloxan induced diabetic rats. *Am. J. Biochem. Biotech.,* 2 (4): 154 – 160.
21. Mann, J.I. (1980). Diet and diabetes. *Diabetologia*, 18: 89 – 95.
22. Masaba, S.C. (2000). The antimalarial activity of some traditional medicinal plants. *Ethiopian J. Health Develop*., 13: 211 – 216.
23. Naidu, R. (1992). Dietary advice for diabetics in Africa. *International Diabeics Digest*, 3 (4): 114 – 118
24. Nwajo, H.U. (2005). Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoproteins and oxidative status in diabetic rat model. *Nig. J. Physiol. Sci.* 20: 39 – 42.
25. Nwanjo, H.U and Nwokoro, E.A. (2004). Anti-diabetic and biochemical effects of aqueous extract of *V. amygdalina* leaf in normoglycaemic and diabetic rats. *J. Innov. Life Sci*. 7:6 – 10.
26. Obi, I.U. (1990). Statistical methods of detecting differences between treatment means. 2nd ed. Snap Press, Enugu – Nigeria.
27. Ohiagashi, M.(1994). Assessment of antibacterial activity of some traditional medicine plants. *Ethiopian J. Health Develop.,*13:211-216
28. Ojewole, J.A.O. (2006). Antinociceptive, anti-inflammatory and antidiabetic effects of *Bryophyllum pinnatum* (crassulaceae) leaf aqueous extract. *J. Ethnopharmacol*. 99: 13 – 19.
29. Ojiako, O.A. and Nwanjo, H.U. (2006). Is *Vernonia amygdalina* hepatotoxic or hepatoprotective. Response from biochemical and toxicity studies in rats. *Afric. J. Biotech.* 5 (18): 1648 – 1651.
30. Owen, O.J.; A.O. Amakiri, E.U. David, V.N. Nyeche and L. Ndor (2009). Proximate composition, energy content and mineral profile of *Vernonia amygdalina* (Bitter Leaf) meal. *Proceedings 14th Annual Conference Animal Science Association of Nigeria (ASAN)*, 14th – 17th September, Ogbomoso, Oyo State. Pp 173 – 176.
31. Rates, S.M. (2001). Plants as sources of Drugs. *Toxican*, 39 (5). 603 – 613.
32. Sonia, B. and Srinirasan, B.P. (1999). Investigation into the anti-diabetic activity of *A. indica. Ind. J. Pharm*. 31:138 – 141.
33. Steel, R.G.D and J.H. Torrie (1980). Principle and procedures ofstatistics. 2nd ed. MacGraw – Hill. Singapore.
34. Taiwo, I.A.; Odeigah, P.G.C and Ogunknmi, L.A. (2009). The glycaemic effects of *V. amaygdalina* and *V. tenoreana* with tolbutamide in rats and implications for the treatment of diabetes mellitus. *Journal Science Research Development*, 11: 122 – 130.
35. Tiwari, A.K. and J.M. Rao (2002). Diabetes mellitus and multiple therapeutic approaches of phytochemical: Present status and future prospects. *Curr. Sci*., 83 (1): 30 – 37.
36. Uhegbu, F.O. and Ogbuehi, K.J. (2004). Effect of the aqueous extract (crude) of leaves of *Vernonia amygdalina* on blood glucose, serum cholesterol and serum albumin levels in alloxan induced diabetic albino rats. *Global J. Pure Appl. Sci.* 10: 189 – 194.
37. WHO (World Health Organization) (1980). Second report of the WHO Expert Committee on Diabetes Mellitus. Technical Report Series 646, Genera, pp: 66.