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Effect of Activity Directed Fractions of *Vernonia amygdalina* on Total Body Weight and Blood Glucose Levels of Diabetic Wistar Albino Rats

[1]Ekam V. S., [1]Ebong P. E., [2]Johnson J. T., [2]Dasofunjo, K.
[1]Department of Biochemistry, College of Medical Sciences University of Calabar,
P.M.B 1115 Calabar, Cross River State Nigeria.
[2]Department of Chemical Sciences, College of Natural Sciences University of Mkar,
Mkar, P.M.B. 017. Benue State Nigeria.

ABSTRACT

The effect of administering activity directed fractions of *Vernonia amygdalina* leaves on diabetic wistar albino rats was investigated on 84 wistar albino rats divided into 14 groups of 6 rats each. Group 1, the normal control was non-diabetic and received only distilled water; Group 2, the diabetic control group, was made diabetic and received only distilled water. Groups 3-14, were induced diabetes mellitus using 150mg/kg body weight of alloxan and treated with various activity directed fractions of *Vernonia amygdalina*. The fractions were obtained by fractionation of the crude ethanolic extract using organic solvents of increasing polarities. The experimental treatment period lasted for 14 days. Two doses of the extracts used were 100mg/kg (dose 1) and 200mg/kg (dose 2) body weight. Treatment with both the 100mg/kg and 200mg/kg doses of the benzene, chloroform and butanol fractions as well as the 100mg/kg dose groups treated with ethyl acetate and residue E fractions resulted in considerable improvements in growth rate and body weight increase. The 100mg/kg doses of benzene, ethyl acetate, methanol and residue E fractions resulted in more improved outcomes in growth rate and weight increase compared to their 200mg/kg dose counterparts. Comparison of the blood glucose levels show that the percentage decrease in blood glucose level was significantly lower in the diabetic control group compared with the normal control. Treatment of the diabetic rats with 100mg/kg and 200mg/kg doses of the benzene fraction caused drastic reductions in the blood glucose levels; with the 200mg/kg dose bringing the blood glucose level to physiological level. In the methanol and butanol as well as residue E fraction treated groups, blood glucose levels reduced significantly ($p<0.05$) within the two weeks treatment period, although this did not normalize the hyperglycemic condition within the limited time (14 days) in which the treatment lasted.

Keywords: *Activity-directed fractions, Vernonia amygdalina, total body-weight ,blood glucose,diabetes*

1. INTRODUCTION

Several scientists all over the world attempted to isolate and purify substances from the pancreas that were supposed to cure diabetes mellitus. Meanwhile, several diabetologists kept believing that a diet was the only good way to treat diabetics. Over the past half century, diabetes has become a major public health concern worldwide. World Health Organization estimates predict a doubling of the number of individuals with diabetes over the next 30 years (King *et al*., 1998). By 2025, it is estimated that there will be approximately 300 million individuals affected by diabetes. Developed countries have traditionally noted higher diabetes prevalence rates; however, over the next 25 years, the largest increases in diabetes prevalence are expected to occur in developing countries where an estimated 170% increase is estimated (WHO, 1989). The 1989 St. Vincent Declaration identified diabetes as a major health problem for Europe that required focused strategies for care, prevention, and research (WHO, 1989). This document was later echoed in North America with the 1996 Declaration of the Americas (PAHO, 1997). Most cases of diabetes mellitus result from decreased insulin secretion (type 1, insulin-dependent) or altered insulin action (type II, insulin-independent). Another category, namely, "other" diabetes mellitus-associated conditions, is usually mentioned to distinguish this type of diabetes from the other two categories; this category includes drugs, genetic and endocrine syndromes, pregnancy and pancreatic disorders. The most common pancreatic disease that causes diabetes mellitus is chronic pancreatitis that results from alcohol abuse (Greenhouse and Lardinois, 1996). Diabetes mellitus is a common condition which frequently has skin manifestations. The attachment of glucose to protein may result in profound effect on structure and function of that protein, and account for clinical manifestation of the disease. It has been suggested that increase cross linking of collagen in diabetic patients is responsible for the fact that their skin is generally thicker than that of non-diabetics (Huntley, 2004). Advanced glycosylation end products are probably responsible for yellowing of skin and nails. Increased viscosity of blood due to stiff red blood cell membranes results in engorgement of the post-capillary venules in the papillary dermis, detected as erythema of the face, or periungual erythema. It is

suggested that these skin changes may eventually be used as a reflection of the patient's current as well as past metabolic status (Huntley, 2004). Before the introduction of insulin in 1922, the treatment of diabetes mellitus relied heavily on dietary measures which included the use of traditional plant therapies. Many traditional plant treatments for diabetes exist (Gray *et al.*, 1997) but few have received scientific or medical scrutiny. Traditional plant remedies continue to have widespread use for the treatment of diabetes in countries where modern medicines are not readily available (Bailey and Day, 1989) and the World Health Organization has recommended that traditional plant therapies for diabetes warrant further evaluation. According to WHO, a diabetic epidemic is underway. An estimated 30 million people worldwide had diabetes in 1985. A decade later, the global burden of diabetes was estimated to be 135 million. With the 2000 estimate, at least 177 million people worldwide suffer from diabetes mellitus and the number is expected to be more than double by 2030 (WHO, 1989). Despite exhaustive efforts to better manage patients with diabetes mellitus, attempts at maintaining near normal blood glucose levels in these patients remain unsatisfactory. In tropical countries, modern medicines are not available to most of the rural populations. The World Health Organization has estimated that 80% of the world's population use ethnomedicines for their primary healthcare needs. Before the introduction of insulin in 1922, the treatment of diabetes mellitus relied heavily on dietary measures which included the use of traditional plant therapies; and many traditional plant treatments for diabetes exist but few have received scientific or medical scrutiny. The World Health Organization (1989) has recommended accordingly and traditional plant treatment for diabetes warrant further evaluation.

2. MATERIALS AND METHODS

2.1 Collection and Treatment of Plant Samples

Fresh leaves of *Vernonia amygdalina* were harvested from the endocrine research farm in the University of Calabar. It was identified in the department of botany, University of Calabar. They were dried under shade, crushed and soaked in 98% ethanol for 24 hours; then filtered and allowed to evaporate at room temperature to obtain the crude extract. The crude extract was subjected to fractionation using organic solvents of varying polarities. It was first soaked in benzene in a separating funnel, shaken and allowed to separate into two fractions. The benzene soluble fraction was obtained and allowed to dry under room temperature to obtain the benzene fraction. The resulting residue (residue A) was dried, and then fractionated using chloroform. The chloroform soluble part was dried at room temperature to obtain the chloroform fraction while the resulting residue (residue B) was dried and subsequently fractionated using ethyl acetate. The ethyl acetate soluble fraction was then dried to obtain the ethyl acetate fraction and an insoluble residue, (residue C). The insoluble residue obtained was again re-suspended in butanol, shaken in a separating funnel, and allowed to separate into two distinct layers. The butanol soluble fraction was dried at room temperature, to obtain the butanol fraction, while the resulting residue (residue D) was dried and further fractionated using methanol. The methanol soluble fraction was dried to obtain the methanol extract, while the insoluble portion was dried to obtain an insoluble residue (residue E) which was soluble in water.

2.2 Laboratory Animals

Eighty four wistar albino rats weighing between 75 to 120g were obtained from the animal house of the Department of Biochemistry, University of Calabar. They were housed in plastic cages in the animal house, and fed with rat pellets and tap water *ad libitum*. The animals were acclimatized for two weeks and their weights noted before the commencement of experimental treatment. The animals were divided into fourteen groups, based on their weights.

Groups 1 and 2 served as the normal control and diabetic control respectively while groups 3 to 14 served as the treatment groups. The animals in group one received tap water throughout the treatment period. Group two animals were induced with diabetes using 150mg/kg of alloxan; and also given tap water orally throughout the treatment period. Animals in groups three to fourteen were induced with diabetes and treated with 100mg/kg and 200mg/kg body weights of the extracts. The various fractions of benzene, chloroform, ethyl acetate, butanol, methanol and residue E were dispersed in distilled water and administered twice a day (12 hours apart) for a period of 14 days. Blood glucose levels were taken three days after induction of diabetes mellitus and at two days intervals thereafter while body weights of the rats were taken every two days throughout the pre-treatment and treatment periods.

3. RESULT

3.1 Effect of Treatment on Total Body Weight

The weight of rats after two weeks of administration of activity directed extracts of *Vernonia amygdalina* were obtained and compared with the initial weights measured before each treatment. The differences in weights were used to determine the percentage weight increases and the growth rates during the period. The result shows a significant difference ($p<0.05$) in both percentage growth rate and weight increase among and within the test groups and control (Table 1). There were significant decreases ($p<0.05$) in both growth rate and weight increase in the diabetic control group (-428.57 ± 4.88 and -40.00 ± 1.09) compared to the normal control (228.57 ± 5.17 and

21.33±1.28) treated with various extracts of *Vernonia amygdalina* using the 100mg/kg and 200mg/kg body weight for two weeks resulted in significant increases ($p<0.05$) in percentage growth rate in all test groups compared to the diabetic control groups. The values were not comparable to the normal control group that was not induced with diabetes; but however were highest in the benzene treated groups that received the low dose (-100.00±2.33) and normal dose (-107.14±3.18) extracts, and lowest in the groups that received normal dose extracts of ethyl acetate (-357.14±3.98), methanol (-357.14±4.00) and residue E (357.14±3.88). Growth rates (%) for both low and normal dose extracts were however non-significantly different ($p>0.05$) in groups receiving extracts of benzene (-100.00±2.33 and -107.14±3.18), chloroform (-178.57±2.56) and -178.57±2.51) and butanol (-178.57±2.22 and -178.57±2.31). The weight increases (%) were significantly increased ($p<0.05$) in all tested groups except the normal dose ethyl acetate treated groups (-33.33±2.97) compared to the diabetic control group (-40.00±1.00) also. Also, results for weight increases (%) were non significantly different ($p>0.05$) in the 100mg/kg and 200mg/kg treated dose except for the residue E (-14.29±1.00 and -28.57±3.21) and ethyl acetate (-20.00±1.56 and 33.33±2.97) treated groups compared to the diabetic control group.

3.2 Effect of Treatment on Blood Glucose Levels

The blood glucose levels (mg/dl) of rats after two weeks of administration of activity directed extracts of *Vernonia amygdalina* were obtained and compared with the blood glucose levels measured three days after the induction of diabetes mellitus using alloxan. The results obtained were used to determine the percentage decrease in blood glucose levels. The results show a significant difference ($p<0.05$) in the percentage blood glucose level among and within the test groups as well as the control groups (table 2). From the result the blood glucose level (mg/dl) for the normal control group increased from 63.72±4.82 to 68.32±3.33 mg/dl over the two weeks period. The 7.22±1.50 percent increase in blood glucose was however within the normal blood glucose range. In the diabetic control group, the blood glucose level (mg/dl) increased from 312.48±6.33, three days after induction of diabetes to 588.00±6.17mg/dl after two weeks; representing 92.31±3.15 percent increase in blood glucose level mg/dl were also recorded for the 100mg/kg and 200mg/kg dose chloroform groups; from 198.56±4.27 to 489±6.50 in the low dose group and from 242.18±4.88 to 308.51±3.81 in the 200mg/kg dose group representing 146.97±4.67 and 27.27±6.15 percent increases in blood glucose levels in these groups. The 200mg/kg dose ethyl acetate group also recorded an increase in blood glucose level (mg/dl) from 208.14±4.54 three days after induction of diabetes to 425.10±5.88 after two weeks of treatment; representing 108.33±6.15 percent increase in blood glucose level. Blood glucose level for all of the other treatment groups were significantly decreased ($p>0.05$) to varying extents. In the benzene extract treated group, the 200mg/kg dose group produced a significantly lower ($p<0.05$) value (47.24±3.68 mg/dl) compared to the 100 mg/kg dose group (82.00±4.08 mg/dl). Both values falling within the normal blood glucose level after two weeks of administration (from 562.00±8.61 to 108±5.29) in the 100mg/kg dose group and from 163.22±4.08 to 86.50±4.30 in the 200mg/kg dose group). In the groups treated with butanol, blood glucose levels decreased significantly ($p<0.05$) from 420.08± to 221.42±4.19mg/dl in the 100mg/kg dose group; representing a 47.38±4.87 percent decrease. In the methanol treatment group, blood glucose level were significantly reduced ($p<0.05$) from 517.36±8.01 to 259.16±4.23mg/dl in the 100mg/kg dose groups representing 49.90±3.96 percent decrease and from 592.00±7.59 to 337.31±4.18mg/dl in the 200mg/kg dose groups representing 43.83±4.52 percent decrease in blood glucose level. For groups treated with residue E. blood glucose level (mg/dl) were decreased ($p<0.05$) from 327.15±5.49 to 257.43±4.59 in the 100mg/kg dose group and from 599.23±7.65 to 243.14±4.65 representing 21.41±2.11 and 59.50±3.64 percent decrease in the two groups.

Table 1. Effect of activity directed extracts of *Vernonia amygdalina* on total body weight of diabetic wistar albino rats

Group	Treatment	Growth rate %	Weight increase %
1	Normal control	228.57±5.17	21.33±1.28
2	Diabetic control	-428.57±4.88[a]	-40.00±1.09[a]
3	Benzene extract I	-100.00±2.33[a,b]	-18.67±1.34[a,b]
4	Benzene extract II	-107.14±3.18[a,b]	-20.00±1.52[a,b]
5	Chloroform Extract I	-178.57±2.56[a,b]	-25.00±1.86[a,b]
6	Chloroform Extract II	-178.57±2.51[a,b]	-20.00±1.59[a,b]
7	Ethyl Acetate Extract I	-178.57±2.54[a,b]	-20.00±1.56[a,b]
8	Ethyl Acetate Extract II	-357.14±3.98[a,b]	-33.33±2.97[a,b]
9	Butanol Extract I	-178.57±2.22[a,b]	-16.67±0.98[a,b]
10	Butanol Extract II	-178.57±2.31[a,b]	-16.67±1.06[a,b]
11	Methanol Extract I	-268.29±4.81[a,b]	-21.14±2.15[a,b]
12	Methanol Extract II	-357.14±4.00[a,b]	-28.57±2.43[a,b]

13	Residue E I	-178.57±2.18[a,b]	-14.29±1.00[a,b]
14	Residue E II	-357.14±3.88[a,b]	-28.57±3.21[a,b]

Results are expressed as Mean + SD
n = 6
a = p<0.05 compared to the normal control group
b = p<0.05 compared to the diabetic control group.

Table 2. Effect of activity directed extracts of *Vernonia amygdalina* on the blood glucose levels of diabetic wistar albino rats

Group	Treatment	Blood glucose level 3 days after induction of DM	Blood glucose levels after 2 weeks of treatment	% Decrease in blood glucose level
1	Normal control	63.72±4.82	68.32±3.33	-7.22±1.50
2	Diabetic control	312.48±6.33[a,b]	588.00±6.17[a,b]	-92.31±3.15[a,b]
3	Benzene extract I	562.00±861[a,b]	108.15±5.29[a,b]	82.00±4.08[a,b]
4	Benzene extract II	163.22±4.08[a,b]	86.50±4.30[a,b]	48.24±3.68[a,b]
5	Chloroform Extract I	198.56±4.27[a,b]	498.00±6.50[a,b]	-146.97±4.67[a,b]
6	Chloroform Extract II	242.18±4.88[a,b]	308.51±3.81[a,b]	-27.27±6.15[a,b]
7	Ethyl Acetate Extract I	564.37±7.63[a,b]	498.33±5.96[a,b]	11.70±2.89[a,b]
8	Ethyl Acetate Extract II	208.14±4.54[a,b]	425.10±5.88[a,b]	-108.33±6.15[a,b]
9	Butanol Extract I	420.08±6.30[a,b]	221.42±4.19[a,b]	47.38±4.87[a,b]
10	Butanol Extract II	302.63±5.12[a,b]	157.33±3.99[a,b]	48.01±4.11[a,b]
11	Methanol Extract I	517.36±8.01[a]	259.16±4.23[a,b]	49.90±3.96[a,b]
12	Methanol Extract II	592.00±7.59[a,b]	337.31±4.18[a,b]	43.83±4.52[a,b]
13	Residue E I	321.15±5.49[a]	257.43±4.59[a,b]	21.41±2.11[a,b]
14	Residue E II	599.23±7.65[a,b]	243.14±4.65[a,b]	59.50±3.64[a,b]

Results are expressed as Mean ± SD
n = 6
a = p<0.05 compared to the normal control group
b = p<0.05 compared to the diabetic control group.

4. DISCUSSION

The results show that induction of diabetes mellitus using alloxan caused a decrease in both growth rate (%) and weight increase (%) in the diabetic rats compared to the normal control group that was not induced. This agrees with earlier reports that diabetes mellitus as a syndrome can cause a significant decrease in growth rate of rats. Treatment of diabetic rats with 100mg/kg and 200mg/kg body weight using the various fractions of activity directed extracts of *Vernonia amygdalina* (viz benzene, chloroform, ethyl acetate, butanol, methanol and residue E) for two weeks resulted in increased percentage growth rate and weight increase in all treatment groups compared to the diabetic untreated group. The improvements in weight increase and growth rate however varied in a dose dependent manner and also depending on the fraction administered. Treatment with both the 100mg/kg and 200mg/kg doses of the benzene extract resulted in considerable improvements in growth rate and weight increase followed by those of chloroform and butanol as well as the 100mg/kg dose groups treated with ethyl acetate extract and residue E. the 100mg/kg doses of extracts of benzene, ethyl acetate, methanol and residue E resulted in more improved outcomes in growth rate and weight increase compared to their 200mg/kg dose counterparts. These suggest that treatment of diabetic rats using various activities directed extracts of *Vernonia amygdalina* can arrest the drastic reductions that occur in growth rate and weight increase evident in the diabetic control group. Comparison of the blood glucose levels in rats induced with diabetes mellitus (diabetic control) with those of the normal control showed that blood glucose levels were increased in the diabetic control group three days after induction of diabetes; and the values continued to be on the increase after two weeks without treatment. The percentage decrease in blood glucose level was significantly lower in the diabetic control group compared with the normal control. This agrees with the report of earlier researches that administration of alloxan is able to induce hyperglycemia in rats and that the condition can be sustained if untreated producing all the symptoms of diabetes mellitus. Alloxan and products of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. There after highly reactive hydroxyl radicals are formed by the Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of beta cells (Sckudelski, 2001). From the results presented in table 2, treatment of the diabetic rats with 100mg/kg and 200mg/kg doses of the benzene extract caused drastic reductions in the blood glucose levels; with the 200mg/kg dose bringing the

blood glucose level to physiological level. Earlier researches had demonstrated that the ethanolic extract of *Vernonia amygdalina* could ameliorate the hyperglycemia caused by diabetes mellitus (Uguwibe, 2004). Treatment with other activity directed extracts also produced varying responses in blood glucose levels. In the methanol and butanol as well as residue E extract treated groups, blood glucose levels reduced significantly ($p<0.05$) within the two weeks treatment period, although this did not normalize the hyperglycemic condition. The inability to completely reverse the hyperglycemia in these groups may be due to the limited time (14 days) in which the treatment lasted. Chloroform and ethyl acetate extracts did not have any anti-hyperglycemic activities as blood glucose levels in these groups continued to rise. The benzene extract was able to keep the blood glucose level within the threshold within the two weeks treatment period.

5. CONCLUSION

The result of treatment of diabetic wistar albino rats with both the 100mg/kg and 200mg/kg doses of the benzene, chloroform and butanol fractions as well as the 100mg/kg dose groups treated with ethyl acetate and residue E fractions resulted in considerable improvements in growth rate and body weight increase. The 100mg/kg doses of benzene, ethyl acetate, methanol and residue E fractions resulted in more improved outcomes in growth rate and weight increase compared to their 200mg/kg dose counterparts. These suggest that treatment of diabetic rats using this activity directed fractions of *Vernonia amygdalina* can arrest the drastic reductions that occur in growth rate and weight increase evident during diabetes mellitus. Comparison of the blood glucose levels show that the percentage decrease in blood glucose level was significantly lower in the diabetic control group compared with the normal control. This agrees with earlier reports that administration of alloxan is able to induce hyperglycemia in rats and that the condition can be sustained if untreated producing all the symptoms of diabetes mellitus. Treatment of the diabetic rats with 100mg/kg and 200mg/kg doses of the benzene extract caused drastic reductions in the blood glucose levels; with the 200mg/kg dose bringing the blood glucose level to physiological level. In the methanol and butanol as well as residue E extract treated groups, blood glucose levels reduced significantly ($p<0.05$) within the two weeks treatment period, although this did not normalize the hyperglycemic condition within the limited time (14 days) in which the treatment lasted.

REFERENCES

[1] Bailey, C. J. and Day, C. (1989). Traditional treatments for diabetes. *Diabetes care.* 12. 553-564.

[2] Gray, A.M. and Flatt, P.R. (1997). Nature own pharmacy: the diabetes perspective. *Proc. Nutritional Society*, 56: 507-577.

[3] Greenhouse, L. and Lardinois, C.K. (1996). Alcohol – Associated Diabetes mellitus. A review of the impact of alcohol consumption on carbohydrate metabolism archives of family medicine, 5 (4), USA.

[4] Huntley, A. (2004). Diabetes in skin diseases. University of California at Davis. 124-156.

[5] King, H., Aubert, R. E. Herman, W.H. (1998). Global burden of diabetes, 1995-2005: prevalence, numerical estimates, and projections. Diabetes care; 21(91): 1414-1431.

[6] Pan American Health Organisation (PAHO) (1997). Declaration of the Americas on Diabetes. Diabetes care. 20:1040-41.

[7] Sckudelski, T. (2001). The mechanism of alloxan and streptozotocin action in beta cells of the pancreas. Physiology Research 50(6): 537-546.

[8] Ugwuibe, G.O. (2004). Preliminary studies on the hypoglycemic effects of *Vernonia amygdalina* on streptozotocin induced diabetes albino wistar rats. (Unpublished results). Department of Biochemistry, University of Calabar, 90-92.

[9] World Health Organization and International Diabetes Federation in Europe (1989). Diabetes care and research in Europe; Saint Vincent Declaration. *British medical Journal* 299 1198-1201.