Biochemical Effects of Single and Combined Ethanolic Leaf Extracts of *Vernonia Amygdalina* and *Ocimum Gratissimum* on Liver and Renal Functions of Alloxan-Induced Diabetic Wistar Rats

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ABSTRACT

To study the effects of single and combined ethanolic leaf extracts of Vernonia Amygdalina (VA) and Ocimum Gratissimum (OG) on liver and renal functions of alloxan-induced diabetic Wistar rats. Thirty (30) albino Wistar rats of body weights between 100g and 150g were randomly distributed into six (6) groups of five (5) animals each and treated for 14 days. Group 1 animals served as normal control, and were fed with normal feed and water ad libitum while group 2 served as the diabetic control. Groups 3 and 4 were treated orally with 200mg/kg body weight of VA and OG respectively while groups 5 and 6 were treated orally with 100mg/kg body weight each of VA and OG and 5mg/kg body weight of insulin respectively. Parameters measured were serum AST, ALT and ALP levels for liver function as well as serum sodium, chloride, potassium, bicarbonate, urea and creatinine levels for renal function. Results showed a significant (p?0.05) reduction in the fasting blood sugar (FBS) level in the treated groups compared with the diabetic control group. There were also significant (p?0.05) reductions in serum activities of AST, ALT and ALP of the treated groups compared with the diabetic control group. Serum chloride and sodium concentrations across all the groups were not significantly altered, however significant (p?0.05) reductions in serum potassium concentrations and increase in bicarbonate concentrations were observed following extract treatment. Treatments also significantly (p?0.05) reduced the serum concentrations of urea and creatinine when compared to the diabetic control. We therefore conclude that single and combined leaf extracts of Vernonia Amygdalina and Ocimum Gratissimum can be a source of new drugs for effective management of diabetes and the associated diabetic nephropathy.

Keywords: Vernonia Amygdalina, Ocimum Gratissimum, Alloxan, Diabetes, Diabetic Nephropathy

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases that is characterized by hyperglycemia resulting from either a lack of secretion of insulin or a decrease in the sensitivity of tissues to insulin or both[1]. According to World Health Organization's (WHO) report in 1999, long-term effects of diabetes mellitus include (amongst others) progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, among others.

Several studies have shown that experimental animal models are very useful in understanding the various physiologically disordered processes associated with different diseases, which may be informative in the production of drugs for the treatment of such diseases[2]. One of the very common substances used to induce diabetes in experimental animals is Alloxan[2].

Alloxan (2,4,5,6-tetraoxypyrimidine; 5,6-dioxyuracil) is a urea derivative which is commonly used for the induction of diabetes in experimental animals like mice, rats and dogs[2,3]. Diabetes is currently being managed by exercise, diet, insulin replacement therapy and by herbal agents that are known to possess hypoglycemic activities[4]. It has been reported that about 800 plants could be successfully used to combat the effects of diabetes mellitus[5].

Vernonia Amygdalina (Bitter leaf), also known as "Etidot" in Ibibio, "Oriwo" in Bini, "Ewuro" in Yoruba, "Shikawa" in Hausa, and "Olubu" in Igbo, all of Nigeria, is a small tree, 1-3m in height with petiole leaf of about 6mm in diameter, and it is elliptic in shape[6]. The leaves are dark green in colour with a characteristic bitter taste and odour, owing to its chemical contents which are likely responsible for its medicinal properties[7]. *Vernonia amygdalina* is commonly used as anti malarial, anthelmintic, antidiarrheal,

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treatment of venereal diseases, gastrointestinal problems and wounds[7]. Reports show that *Vernonia Amygdalina* is of high medicinal value as it is useful in maintaining the health of the liver and kidney. It is also very helpful in the management of diabetes due to its ability to cause a reduction in blood sugar drastically and its ability to repair the pancreas[8-10].

Ocimum Gratissimum (Scent leaf) also called "Ntong" in Ibibio, "Ebavbokho" in Bini, "Nchonwu" in Igbo, all of Nigeria, is a local plant (and it is) widely utilized for both nutritional and medicinal purposes. It is a fully developed flowering plant with leaves, stem and root[11]. It is known that this plant is used in the treatment of several diseases like skin diseases, pneumonia, diarrhea, headache, fever, eye diseases[12,13]. Reports also show that the extracts from this plant are used the management of diabetes mellitus[14,15]. This study was therefore aimed at comparing the antidiabetic effects of *Vernonia Amygdalina* and *Ocimum Gratissimum as well examining the effect of combining the two plants in the management of diabetes.*

MATERIALS AND METHODS

Collection and Preparation of Plant Extracts

Fresh leaves of *Ocimum gratissimum* were obtained from Ikot Abia Idem, Ikot Ekpene, while *Vernonia amygdalina* leaves were obtained from Ikot Abasi in Akwa Ibom State of Nigeria, identified and authenticated in Botany Department of the University of Uyo, Nigeria. The leaves were then washed to remove debris and contaminants, air dried and then reduced to powdered form by grinding using Qlink blending machine QBL-15L40 from China. The ground leaves were then suspended in 97% ethanol for about 72hours, filtered and then concentrated using a rotary evaporator. The extracts obtained were used for the experiments.

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grades from BDH Merck Ltd., USA and the reagent kits were obtained from Randox.

Experimental Animals

Thirty (30) albino *Wistar* rats were procured from the animal house of the Faculty of Basic Medical Sciences, University of Uyo and then housed in cages to acclimatize for two (2) weeks. The experimental animals were maintained under standard laboratory conditions with rat chow (Guinea feeds Ltd., Nigeria) and water *ad libitum.*

Induction of Diabetes

Prior to the induction of diabetes, the rats were fasted for about 12 hours and diabetes was then induced by a single intraperitoneal injection of 150mg/kg body weight of alloxan monohydrate. Diabetes was confirmed by using a one-touch glucometer and after 72 hours of alloxan induction, rats with plasma glucose levels of = 200mg/dl were considered diabetic and used as diabetic rats for the study.

Experimental Design

Thirty (30) albino wistar rats were randomly divided into six (6) groups of five (5) animals and the experiment lasted for 14 days.

Group 1: Fed with standard feed and water *ad libitum* **(Normal Control)**
Group 2: Diabetic rats that received normal feed and water *ad libitum* **(Diabetic Control)**
Group 3: Diabetic rats treated with 200mg/kg body weight VA once daily for 14 days
Group 4: Diabetic rats treated with 200mg/kg body weight OG once daily for 14 days
Group 5: Diabetic rats treated with 100mg/kg body weight each of VA and OG once daily for 14 days
Group 6: Diabetic rats treated with 5mg/kg body weight insulin once daily for 14 days

Collection of Samples for Biochemical Analyses

At the end of the 14 days of animal treatment, the rats were fasted for about 12 hours and they were then anesthesized using chloroform and sacrificed. Whole blood was collected by cardiac puncture and transferred into plain sample bottles to clot for about two hours. This was then centrifuged at 3000 rpm for about 10 minutes in order to recover the serum which was separated using sterile syringes and then used for the biochemical analyses.

Statistical Analysis

Data were analyzed with student t-test using SPSS statistical package to compare the means between experimental groups and controls. All data were expressed as mean ± Standard deviation

(SD) and data with $p<0.05$ were considered significant.

RESULTS

Effect of Treatment on Blood Glucose Levels of Experimental Rats

Treatment of the diabetic rats with the ethanolic extracts of *Vernonia Amygdalina* (VA) and *Ocimum Gratissimum* (OG) significantly ($p<0.05$) reduced blood glucose levels previously increased by alloxan induction, (as seen when the treatment groups were) compared with the diabetic control group. Of particular interest is the fact that the combined extracts was more efficacious in reducing blood glucose level than the extracts administered separately and insulin treatment.

Effect of Treatment on Liver Enzymes

Treatment of the diabetic rats with the ethanolic extracts of *Vernonia Amygdalina* (VA) and *Ocimum Gratissimum* (OG) significantly $p<0.05$ reduced the serum enzyme activities of AST, ALT and ALP when the treated groups were compared with the diabetic control group.

Effect of Treatment on Renal Function Parameters

Administration of alloxan caused non - significant ($p<0.05$) alterations in sodium and chloride levels but however resulted in significant increase in potassium, urea and creatinine; and decrease in bicarbonate concentrations. Treatment of the diabetic rats with ethanolic extract of *Vernonia Amygdalina* (VA) and *Ocimum Gratissimum* (OG) restored the electrolytes, urea and creatinine levels to the

Table 1: Effect of Treatment on Blood Glucose Levels of Experimental Rats

Experimental Group	Blood glucose concentration (mg/dl)
1. Normal control	89.25±9.97
2. Diabetic control	310.50±53.05
3. DVA (200mg/kg bw)	76.75±7.41
4. DOG (200mg/kg bw)	76.75±6.38
5. DVAOG (100mg/kg bw each)	67.50±5.06
6. DI (5mg/kg bw)	93.00±7.25

DVA= Diabetic treated with *Vernonia Amygdalina* (VA)
DOG= Diabetic treated with *Ocimum Gratissimum* (OG)
DVAOG= Diabetic treated with *Vernonia Amygdalina* (VA) and *Ocimum Gratissimum* (OG)
bw= Body weight, DI = diabetic rats treated with insulin. * = significantly higher with $p<0.01$

Table 2: Effect of Treatment on Liver Enzymes

Experimental Group	AST (iu/L)	ALT (iu/L)	ALP (iu/L)	AST/ALP (iu/L)
1. Normal control	108.20 ± 10.75	99.40 ± 11.02	106.60 ± 10.67	1.09 ± 0.41
2. Diabetic control	186.00 ± 13.16	192.20 ± 10.75	207.40 ± 19.20	0.97 ± 0.38
3. DVA (200mg/kg bw)	109.80 ± 15.40	113.00 ± 13.26	119.40 ± 10.49	0.97 ± 0.45
4. DOG (200mg/kg bw)	110.00 ± 10.63	104.60 ± 16.05	99.60 ± 10.49	1.05 ± 0.56
5. DVAOG (100mg/kg bw each)	100.40 ± 1 0.42	99.40 ± 10.49	100.00 ± 13.63	1,01 ± 0.53
6. DI (5mg/kg bw)	103.80 ± 5.08	114.60 ± 10.49	117.20 ± 14.40	0.91 ± 0.27

DVA= Diabetic treated with Vernonia Amygdalina (VA)
DOG= Diabetic treated with Ocimum Gratissimum (OG)
DVAOG= Diabetic treated with Vernonia Amygdalina (VA) and Ocimum Gratissimum (OG)
bw= Body weight , DI = diabetic rats treated with insulin * = significantly higher with $p<0.05$

Table 3: Effect of Treatment on Renal Function Parameters

Experimental group	Sodium (MEq/l)	Cl^- (MEq/l)	K^+ (MEq/l)	HCO_3^- (MEq/l)	Urea (mg/dl)	Creatinine (mg/dl)
1. Normal control	130.10± 15.31	93.50±11.26	4.15±0.45	22.35±0.89	24.73±6.00	0.67±0.02
2. Diabetic control	102.53±12.32	68.25±7.41	5.66±0.43	18.10±2.31	41.49±6.54	1.04±0.59
3. DVA (200mg/kg bw)	115.10±11.35	75.25±9.10	4.08±0.33	21.34±4.78	28.92±8.55	0.78±0.10
4. DOG (200mg/kg bw)	122.67±17.56	80.00±8.44	4.04±0.68	21.47±2.52	21.94±11.22	0.82±0.05
5. DVAOG (100mg/kg bw each)	124.97±18.32	81.75±8.01	3.84±0.34	21.24±5.47	31.12±1.72	0.76±0.03
6. DI (5mg/kg bw)	120.65±15.88	77.75±2.21	4.49±0.68	22.12±3.91	36.30±13.51	0.98±0.14

DVA= Diabetic treated with *Vernonia Amygdalina* (VA)
DOG= Diabetic treated with *Ocimum Gratissimum* (OG)
DVAOG= Diabetic treated with *Vernonia Amygdalina* (VA) and *Ocimum Gratissimum* (OG)
bw= Body weight , DI = diabetic rats treated with insulin * = significantly higher with $p<0.05$

control range. The extracts were observed to perform slightly better than insulin in this regard.

DISCUSSION

Diabetes mellitus is one major terminal disease that has succeeded in eroding the health of so many victims all over the world and it has led to the premature deaths of such victims. This bad omen has led to serious scientific efforts being made in order to combat and win this war against diabetes mellitus. Several in-vivo models such as streptozotocin, diazoxide, and alloxan-induced diabetic rats have been are used in the investigation of medicinal plants with that have suspected hypoglycemic potentials[16,17]. In the present study, we induced diabetes by intraperitoneal injection of alloxan at a single dose of 150mg/kg body weight in *Wistar* rats.

Induction of diabetes with alloxan significantly ($p<0.05$) increased the blood glucose concentrations of the rats when the diabetic control group was compared with the normal control. This probably is as a result of the toxic effect of alloxan on the beta cells of the pancreas by the production of free radicals, oxidation of sulphydryl groups, inhibition of the enzyme glucokinase, and disturbance of calcium homeostasis[18,19].

Increased serum levels of aminotransferases such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are a common indication of hepatic damage and they are observed frequently among diabetics in the general population[20,21]. Free radicals which cause oxidative stress are known to affect liver health and ultimately cause liver damage that can lead to elevated levels of these aminotransferases and other liver enzymes. In this study, it was observed that diabetes induced by alloxan significantly ($p<0.05$) increased the serum activities of AST and ALT which are an indication of liver damage. It was however also observed that the ethanolic leaf extracts of *Vernonia Amygdalina* and *Ocimum Gratissimum* were able to significantly ($p<0.05$) reduce the activities of these enzymes probably by combating the generation of free radicals induced by alloxan since alloxan induces diabetes is by the generation of free radicals[18]. This corroborates the findings of Mohammed *et al.*,[22] and Yeap *et al.*[23]. The action of these plants may be due to the fact that they possess some very important phytochemicals that have been shown to possess antioxidant activities. Studies have shown that these plants possess antioxidant properties and phytochemicals like flavonoids, alkaloids, saponins tannins, and steroids[24-26]. Alkaline phosphatase (ALP) activity which was

initially increased by the induced diabetes was however reduced significantly ($p<0.05$) by these plants extracts.

Diabetes mellitus causes damage, dysfunction and failure of the kidneys and this condition is known as diabetic nephropathy. Serum levels of urea, creatinine and some electrolytes are major biochemical parameters used to assess the health of the kidneys. From our study, both *Vernonia Amygdalina* and *Ocimum Gratissimum* leaf extracts when administered separately and in combination significantly ($p<0.05$) reduced the serum levels of urea and creatinine. However, the combined administration of both extracts showed similar effects as when they were administered singly. This may also be due to the antioxidant properties of these plants in combating oxidative stress as the kidneys are also affected by free radicals[24-26]. The extracts had no significant ($p<0.05$) effects on the serum sodium and chloride levels but levels of potassium ions were significantly ($p<0.05$) reduced and bicarbonate ions increased when the treated groups were compared with the diabetic control.

Diabetic nephropathy is one of the major microvascular complication of diabetes mellitus. Its pathogenesis has been associated with increased protein glycosylation and generation of reactive oxygen species capable of altering the structure and function of essential cellular macromolecules, including those of the glomerular basement membrane. The antioxidative properties of the extracts of *Vernonia Amygdalina* and *Ocimum Gratissimum* leaves occasioned by their phytochemical contents[27] are believed to be responsible for the reversal of impaired kidney functions. In addition, the extracts reduced the blood glucose levels from those seen in diabetic conditions to normal levels, probably by quenching reactive alloxan intermediates and enhancing pancreatic β-cells regeneration and insulin levels. Because Careful blood glucose control in diabetics is known to reverse early changes in glomerular function[28] and other complications,[29]. The reduction of blood glucose levels in diabetic rats by the extracts may be a significant factor in the restoration of liver and renal functions observed in this study.

CONCLUSION

In conclusion, ethanolic leaf extracts of *Vernonia Amygdalina* and *Ocimum Gratissimum* show comparable protective potentials against diabetes mellitus by reducing blood sugar concentration and by improving on the integrity of the liver and kidneys. Also, a combination both extracts does not have any additional advantage in protecting against diabetes mellitus. Therefore, *Vernonia Amygdalina* and *Ocimum Gratissimum* may be effectively utilized in the management of diabetes mellitus after appropriate standardization of dosage regimen.

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