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BIOCHEMICAL EFFECT OF VARIED MIXTURES OF EXTRACTS OF *Vernonia amygdalina* (Bitter leaf) AND *Gnetum africanum* (Okazi leaf) ON ALLOXAN INDUCED DIABETIC WISTAR RATS

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ABSTRACT

*Diabetes mellitus is prevalent in many countries of the world, affecting all ages both in developing and developed nations. The use of plants as remedies or preventive therapies has increased over the years. The study investigated the biochemical changes caused by combined leaf extracts of Vernonia amygdalina (bitter leaf) and Gnetum africanum (okazi leaf) on alloxan induced diabetic wistar rats.Aqueous extracts of the leaves were prepared using the conventional method.Forty male wistar rats weighing 150-180g were grouped into eight (five rats each). Group 1 was the normal control while diabetes was induced using alloxan (160mg/ kg)in groups 2-8.Group 2 received no treatment while groups 3-7 received varied ratios of the extracts at (BI/OK|10:90%), (BI/OK|30:70%), (BI/OK|50:50%), (BI/OK|70:30%) and (BI/OK|90:10%). Group 8 was the diabetic control treated with the standard diabetic drug (Metformin). T*he animals were weighed and blood glucose was determined at 7-day intervals. *They were sacrificed on the 28th day and blood samples collected for serum protein, serum electrolyte, urea, creatinine, liver enzymes and markers of oxidative stress analyses. The results showed steady increase in the body weights (g) of the rats with (BI/OK|70:30)% treated group showing the highest increase (175.40±1.28). The fasting blood sugar (mg/dl) showed time-dependent reduction in all the treated diabeti groups with (BI/OK|90:10)% having the highest (56.20±1.65) reduction. There was a significant increase ($p<0.05$) in total blood protein concentration (g/dl) in all the treated groups. The results of this study showed time and ratio dependent effect on the parameters measured. Since the two plants are staple vegetables in some countries, their utilization particularly in appropriate combinations should be encouraged.*

Key words: Extracts, bitter leaf, waterleaf, diabetes mellitus, biochemical parameters.

INTRODUCTION

Diabetes mellitus (DM) is described as a disease syndrome with an associated metabolic disorders characterized by chronic hyperglycemia which results from alterations in insulin secretion, insulin action, or both (American Diabetes Association(ADA), (2014)) and (Groop and Pociot ,(2014)) These metabolic disorders include, but are not limited to alterations in the breakdown of major energy molecules - carbohydrates, fats, and proteins that usually culminate in risks of diabetes (Prince and Kamalakkannan, (2006)). Alterations in the activities of some proteins or enzymes of glucose metabolisms or transport in target tissues such as liver, muscle and adipose tissues cause changes in

the metabolism of the energy molecules. Glucose is the form in which sugar is absorbed through the intestinal tract and moved round the body. In the liver, glucose is stored as glycogen and as starch in plants and seeds (Nelson and Cox, 2005). Glucose is the major source of energy in animals. It has been estimated that brain cells for instance, need over 120 grams of glucose for its performance everyday (Garrett and Grisham, 2010). The body has tightly controlled mechanisms to keep the blood sugar level (BSL) normal. The BSL increases when foods containing carbohydrates such as cereals, yam, cassava, Potatoes, bread, pasta, cakes and sweets are consumed. When digested, carbohydrates are split into sugar and absorbed into the bloodstream, leading to rise in BSL. Insulin, a peptide hormone secreted by the β cells of the pancreatic islets of Langerhans maintains normal blood glucose levels by facilitating cellular glucose uptake. A rise in blood glucose level stimulates pancreatic β-cells to sense the increase in glucose level and then to secrete insulin in an amount that is appropriate ((Meglasson and Matschinsky (1986), De and Saudubray (2000)).This amount maintains the normal glucose level while allowing the excess to be stored in the liver as glycogen. The glycogen is converted to glucose via glycogenolysis when the blood glucose level falls below the normal range. Type 2 diabetes arises when the β cells fail to sense the increase in glucose, which leads to faulty regulation of appropriate amount of insulin in the blood (Gerich,(2003)).The result of which is higher-than-normal blood glucose level often referred to as hyperglycaemia which is one of the classical symptoms of diabetes mellitus.

Globally, the prevalence of diabetes mellitus (DM) has reached epidemic levels especially in low and middle income countries. According to the 2019 International Diabetes Federation (IDF) estimates,in 2000, the global estimate of adults living with diabetes was 151 million. By 2009 it had grown by 88% to 285 million. Today, we calculate that 9.3% of adults aged 20–79 years – a staggering 463 million people – are living with diabetes. A further 1.1 million children and adolescents under the age of 20, live with type 1 diabetes.A decade ago, in 2010, the global projection for diabetes in 2025 was 438 million. With over five years still to go, that prediction has already been surpassed by 25 million. IDF estimates that there will be 578 million adults with diabetes by 2030, and 700 million by 2045.(IDF,2019).Africa fared better in the prevalence of DM among the regions. However, undiagnosed diabetes has regional differences in its prevalence with the highest proportion (59.7%) occurring in the African region and global projections show that it will experience the greatest future increase in the burden of DM of about 156% by 2045 [Kibirige et al.,(2019)]. The picture in Nigeria is not different. Nigeria's diabetes mellitus (DM) incidence and prevalence is currently on the rise.

DM is mainly of two types: Type I or insulin-dependent diabetes mellitus (IDDM which begins early in life as a result of inadequacy of insulin due to pancreatic cells alteration. IDDM needs insulin therapy to manage the hyperglycemia. Type II or Non-insulin dependent diabetes mellitus (NIDDM) which is the most common form of DM is common among adults and so usually referred to "Adult

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onset DM. The conventional treatment for DM is administration of insulin to allow the diabetic reach nearly normal carbohydrate, fat and protein metabolism (Nelson and Cox, 2005). There are several drugs like sulfonylureas, biguanides, intravenous insulin injection andother pharmaceutical preparations employed to control DM. Exercise and diet are also integrated into the management protocol.These methods can be expensive and often have unfavorable side effects.There is therefore a need for alternative therapies that will fill the gap created by these treatments methods.World Health Organization(WHO) (1985, 1994) prescribed the use of alternative therapy, mostly in nations where right to conventional regulation procedures is inefficient or inadequate.Plants or rather medicinal plants have remained a significant alternative source of drugs for majority of populations that experience inadequate contacts with orthodox healthcare facilities. Plants with medicinal activities have remained a vital alternative means of drugs to mostly developing nations where modern hospitals may be limited and modern drugs are quite costly.They have always been the preferred alternative source of remedies for prevention and treatment of diseases and ailments. This study investigated the antidiabetic effect of two staple vegetables, bitter leaf (*Vernoniaamygdalina*) and okazi leaf (*Gnetumafricanum*) extracts on alloxan induced diabetic albino rats. *Gnetumafricanum* (GA) is popularly consumed in various forms in Africa especially in Southern Nigeria due to its palatability and taste (Ekpo, 2007). GA, a lone genus and a member of the family Gnetaceae is a dioecious, wild lianas that grows on trees in the humid forests of Africa (Mialoundama, 1993). It has elliptic leaves lined with reticulate veins, resembling those of a dicotyledonous angiosperm. Its leaves are used in the management of enlarged spleen, sore throat and as a cathartic (Burkill, 1994). It is also used to ameliorate nausea and neutralize some poisons. It helps to manage boils and warts when applied externally and ameliorates the pain of childbirth. The leaves of GA can be eaten raw or used in preparing soups and stews (Burkill, 1994). Its medicinal and culinary usage underscore its value as a major dietary supplement with potential biological effects.*Vernonia amygdalina* (VA) known commonly as bitter leaf is found in tropical Africa and has been domesticated in some places in Nigeria. It is found as a herb or shrub with a height of about 1-3m tall in Madagascar and Asia (Izevbigie *et al*., 2004; Ojiako and Nwanjo, 2005) and a member of the plants family, the compositae. In Nigeria, it is called "Oriwo" by the Edo people, Hausa “Chuserdek”, Yoruba “Ewuro”, and the Ibos “Onugbu”. Studies done on the nutritional composition of *V.*ashow that it is rich in minerals such as phosphorus, calcium, potassium, magnesium, zinc, iron and some vitamins such as vitamin A, C and E..Pharmacological studies have reported antihyperglycemic effects of the roots. Hypolipidemic and antihyperlipidemic, nephron and hepato protective properties ((Ijeh and Obidoa, 2004, Atangwho et al., 2007, Ijeh and Ejike, 2011). It was used to treat diseases such as eczema, measles and anemia in folkloric medicine (Akinpelu, 1999; Ohigashi et al., 1991). This study evaluated the effect of administering these varied combinations of bitter leaf (*vernonia amygdalina*) and okazi leaf (gnetum

africanum).extracts on some biochemical parameters and body weight inWistar rats.

MATERIALS AND METHODS

Bitter leaf and Okazi leaf were purchased from Choba market Port Harcourt and identified by Dr. Chimezie Ekeke of the department of Plant Science and Biotechnology of the University of Port Harcourt, Rivers State, Nigeria.Ethical conditions governing the conduct of experiments with life animals were strictly followed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised in 1996). Forty (40) male Wistar rats weighing 150-180g and bred at the animal house of university of Port Harcourt, Rivers State were used. The rats were kept in stainless steel cages and allowed water and feed *ad libitum*. After 1 week of acclimatization, the treatment commenced and lasted for 28 days.

Preparation of the extract was done using the method of Akah and Okafor (1992) .The leaves were washed and shade- dried for 7 days. They were pulverized into powder using electric blender.Distilled water was added to the leaf powders. Then, the mixture was filtered using Whatman No.1 filter paper. The filtrate was concentrated using water bath at a temperature of 50 °C; then evaporated to dryness to give a dark green paste. The paste was reconstituted for the experiment. Forty Wistar rats were grouped into eight of five rats each. Diabetes was induced in the experimental animals of groups 2-8 while group 1 served as normal control. Upon establishment of diabetes, they were administered with the extract combinations ((BI/OK|10:90%), (BI/OK|30:70%), (BI/OK|50:50%), (BI/OK|70:30%) and (BI/OK|90:10%)) orally daily for 28 days.While metformin was administered daily at 250 mg/kg body weight to the diabetic control group via the same route. The body weights and FBG levels of the animals were measured at seven day intervals. On the 28th day, the animals were sacrificed and blood sample collected for biochemical analysis (protein, serum electrolyte, urea, creatinine, liver enzymes and markers of oxidative stress).. The serum total protein, creatinine and urea were determined using Randox kits. The sodium and chloride ions concentrations were evaluated with the Atlas Medical kits. The colorimetric technique was used for the estimation of liver enzymes. Glutathione reduced (GSH) and catalase were determined by the technique of Lindsay and Beer and Sizer respectively.Data collected were subjected to analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Changes in the body weight of the experimental animals are presented in table 1. Results reveal a progressive increase in the body weights (g) of the rats in all the groups over the 28- day experimental period. Among the treated groups,BI/OK|70:30 showed the highest increase (129.60±0.50 to175.40±1.28) while group 2, negative control showed the least increase (131.80±0.86 to 149.20±0.86).However there was no statistical difference($p< 0.05$) in the weight increment among the groups on the last week of the experimental period except for thenormal control group.Several authors have observed similar trend in the body weight of diabetic rats administered plant extracts (Oyedemi *et al*., (2011), Obasi et

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al., (2019)).Body weight is part of the anthropometric measurements that gives information on the physical state or growth rate of the experimental subject. Weight increment is an evidence of glucose catabolism and consequent biosynthesis of protein and fat (Yakubu *et al*.,(2014)).The comparability of the body weight variations among the extracts group and the metformin group showed that the extracts combinations supported growth the same way as the standard drug for diabetes mellitus.

Table 1: Effect of the combined leaf extracts on the body weight of rats.

GROUP	DAY 0 (g)	DAY 7 (g)	DAY14 (g)	DAY21 (g)	DAY28 (g)
NORMAL	138.20±0.37 ^{a}	149.20±0.58 ^{a}	159.20±0.66 ^{a}	162.00±0.44 ^{a}	169.00±1.04 ^{a}
NEGATIVE CONTROL	131.80±0.86^{b}	135.40±0.92 ^{b}	140.80±1.31^{b}	144.80±1.01 ^{b}	149.20±0.86 ^{b}
BI/OK|10:90	130.40±0.81^{b}	146.40±0.60 ^{a}	158.00±0.70 ^{a}	160.00±0.54 ^{a}	173.40±1.12 ^{a}
BI/OK|30:70	136.20±1.77 ^{a}	148.60±0.67 ^{a}	159.00±1.04 ^{a}	159.60±2.31 ^{a}	173.20±1.15 ^{a}
BI/OK|50:50	129.00±0.70 ^{b}	140.40±1.40^{a,b}	151.40±1.32 ^{a}	152.60±2.56^{b}	166.60±1.66 ^{a}
BI/OK|70:30	129.60±0.50^{b}	141.00±0.70^{a,b}	157.40±1.96 ^{a}	162.40±1.02 ^{a}	175.40±1.28 ^{a}
BI/OK|90:10	139.00±0.44 ^{a}	149.80±0.37 ^{a}	161.40±0.67 ^{a}	163.40±0.97 ^{a}	174.40±1.16 ^{a}
STANDARD DRUG (METHFORMIN)	129.60±0.50^{b}	142.40±0.81^{a,b}	153.80±0.58 ^{a}	157.00±0.54 ^{a}	170.20±2.15 ^{a}

Values are means ± standard deviations for 5 rats per group (n = 5)
Values in the same column with similar superscript letters are not significantly different at 5% level ($p< 0.05$).LEGEND::BI-Bitter leaf;OK-Okazi

Table 2 shows the effect of the different combinations of the extract on the blood glucose of the experimental animals. Administration of the combined extracts to the animals produced significant ($P < 0.05$) decrease in the blood glucose of the animals over the period of the experiment with the highest reduction observed on the 28th day of treatment. Group treated with the ratio BI/OK|90:10 showed the highest reduction from 230.80±23.00 to 56.20±1.65 while the group treated with the ratio BI/OK|30:70 showed the least reduction from 209.80±14.93 to 120.60±4.75.The trend of the reduction was similar to that of metformin (standard drug) group.

Table 2: **Effect of the Combined Extracts on the Fasting Blood Glucose (FBG)**

GROUP	DAY0 (mg/d/L)	DAY7 (mg//L)	DAY14 (mg//L)	DAY21 (mg/dL)	DAY 28 (mg/dL)
NORMAL CONTROL	91.40±3.15^{b}	94.80±2.17^{b}	92.40±4.41^{b}	95.00±1.30^{b}	93.60±3.61^{b}
NEGATIVE	279.20±36.88 ^{a}	301.20±28.84 ^{a}	281.60±16.07 ^{a}	258.20±11.49 ^{a}	171.20±6.01 ^{c}
BI/OK |10:90	218.20±11.33 ^{a}	195.00±10.82^{a,b}	180.40±10.70^{a,b}	164.00±9.23^{a,b}	118.00±6.71 ^{b}
BI/OK |30:70	209.80±14.93 ^{a}	201.00±14.07^{a,b}	181.60±12.56^{a,b}	162.20±11.77^{a,b}	120.60±4.73^{a,b}
BI/OK| 50:50	209.80±18.87 ^{a}	178.20±15.47^{a,b}	130.60±9.81^{b}	105.00±3.24 ^{b}	86.00±3.30 ^{b}
BI/OK |70:30	231.60±25.52 ^{a}	177.80±27.88^{a,b}	143.40±20.65 ^{b}	106.20±13.42 ^{b}	81.00±9.25 ^{b}
BI/OK|90:10	230.80±23.00 ^{a}	177.60±20.77^{b}	131.40±17.59 ^{b}	90.80±10.67^{b}	56.20±1.65^{ab}
METFORMIN .	284.20±36.21 ^{a}	221.60±29.43 ^{a}	148.00±18.47^{b}	109.60±14.82 ^{b}	69.40±8.83 ^{b}

Values are means ± standard deviations for 5 rats per group (n = 5)
Values in the same column with similar superscript letters are not significantly different at 5% level ($p< 0.05$). LEGEND: BI-Bitter leaf; OK-Okazi

This result corroborates with the findings of other workers, who had consistently reported that the leaf extract from the plants possess antidiabetic properties (Akah and Okafor, (1992); Okafor,(1997); Nwanjo *et al*.,(2004),Uhuegbu and Ogbechi,(2004),Ijeh *et al.,* 2013, Kumar.,(2013), Khattab *et al*.,(2013) and Kazeem and Ayobola (2020)). The general reduction in blood glucose level may be as a result of the combined action of the anti-hyperglycaemic and hypoglycaemic activities of some of the phytochemical components of aqueous leaf extract of *Vernonia amygdalina* and *Gnetum afrcanum.* Phytochemical constituents available in extracts were flavonoids, saponins, polyphenols, saponins, sesquiterpene lactones, sterols, glycosides, and tannins Igile *et a*l. (1995),Nnam *et al*.,(2012), Okerulu and Onyema,(2015) Nwaoguikpe,(2010). The flavonoids and polyphenol components of the extracts are well known antioxidants (Tiwari and Rao, 2002). Some plants are reported to owe their antidiabetic effect to the presence of flavone glycosides present in them. Apart from these antioxidant properties, phenols are reported to inhibit alpha (α-) amylase, sucrase, as well as the action of sodium glucose transporter (S-GLUT-1) of the intestinal brush border cells, hence their antidiabetic action (Tiwari and Rao, 2002). It has also been reported that isoflavones, tannins, chlorgenic acids and crude saponins have been found by some workers to possess potent S-GLUT-1 mediated inhibition of glucose transport (Atangwho et al.,(2009)), Krishnamurthy and Asha (2011)). Efficient blood glucose management is the key to hindering or inverting diabetic conditions and enhancing the standard of life in patients with diabetes mellitus. Thus sustained decrease in hyperglycemia will lower the risk of more vascular complications.

The results of the kidney function test and serum electrolytes are presented in table 3.The electrolytes Na^+ and Cl^- (mmol/L) had decreased values in the extract groups which were comparable to the values of methformin group ($P < 0.05$). Plasma electrolyte concentrations reflect the ion composition of interstitial fluid and therefore, can be used to distinguish electrolyte alterations involving the cell interior (Olson *et al*. (1974). This ability of the extracts to modulate plasma electrolytes may be due to the presence of some phytochemicals in them which improves mineral pool distribution in plasma (Rodriguez de Sotillo and Hadley, 2002). The results showed the concentrations of serum urea and creatinine, 4.00±0.08 and 59.80±3.12 respectively in the metformin group which compared favorably ($P < 0.05$)with some of the extract groups. Creatinine is an endogenous substance used as a marker for the determination of kidney function via the glomerular filtration rate (GFR). GFR is inversely related to creatinine level. The amount of this substance in the glomerular helps to ascertain the integrity of the kidney (Peake and Whiting,2006). Urea is a primary metabolite derived from dietary protein and tissue protein turn over. Urea level along with creatinine work hand in hand to determine the glomerular filtration rate of an individual that is to large extent determine the functioning of the kidney (Peake and Whiting, (2006)). An increase in the serum levels of urea and creatinine in clinical analyses presupposes renal dysfunction.Rising serum creatinine and urea is an established indicator of poor glomerular filtration and has been

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established as a significant clinical marker for kidney dysfunction and loss of kidney integrity.The comparable difference in the serum urea and creatinine levels of some of the extract-treated groups and the diabetic control in the study simply showed that the extract combinations had positive effect on the kidneys which agrees with the study of Atangwho *et al., (*2007). This presupposes that the extracts preserved the renal tubular mass as well as its regulatory roles. It also proved that there was no form of abnormality in the metabolism of protein molecules in the muscles following the treatment with the extracts. This is because, creatinine is a major catabolic product of protein metabolism in the muscle tissue and usually excreted by the kidneys.

Table 3: Effect of combined extracts on kidney functions, serum electrolytes and total protein

	Na^+ (mmol/L)	Cl^- (mmol/L)	UREA (mmol/L)	CRE (µmol/L)	T.P (g/L)
NORMAL CONTROL	138.60±2.84 ^{a}	48.80±1.74^{b}	4.24±0.17^{a,b}	63.60±3.18^{b}	63.40±2.83^{b}
NEGATIVE CONTROL	140.80±2.40 ^{a}	57.40±1.72 ^{a}	7.48±0.19 ^{a}	105.20±3.18 ^{a}	50.40±1.86 ^{a}
BI/OK |10:90	116.80±1.20^{a,b}	43.60±0.50^{a,b}	7.38±0.35 ^{a}	81.60±3.90^{a,b}	74.20±1.95^{b}
BI/OK |30:70	105.20±5.40^{a,b}	43.40±0.92^{a,b}	7.14±0.31 ^{a}	93.60±3.07 ^{a}	70.00±1.41^{b}
BI/OK |50:50	119.00±1.00^{a,b}	42.60±0.74^{a,b}	4.12±0.21^{b}	76.20±2.43^{b}	83.20±3.45^{b}
BI/OK |70:30	111.20±1.82^{a,b}	45.20±0.37 ^{b}	6.64±0.35 ^{a}	59.80±3.12 ^{b}	62.80±2.59 ^{b}
BI/OK |90:10	117.80±0.91^{a,b}	42.00±0.44^{a,b}	6.16±0.22^{a,b}	74.20±1.77 ^{b}	74.40±2.83^{b}
STANDARD DRUG (METHFORMIN)	138.60±5.46 ^{a}	42.40±0.50^{a,b}	3.24±0.15 ^{b}	56.80±1.82 ^{b}	80.40±1.63^{b}

Values are means ± standard deviations for 5 rats per group (n = 5)
Values in the same column with similar superscript letters are not significantly different at 5% level ($p< 0.05$).LEGEND:BI-Bitter leaf;OK-Okazi;CRE-Creatinine;TP-Total Protein.

A significant ($P < 0.05$) increase was observed in total protein (TP) (g/L) in all the extract groups and the diabetic control treated group when compared to the negative control.This indicated that the treatments were able to restore protein synthesis which a vital function of the liver in the groups because inhibition of protein synthesis manifests as decrease in serum total proteins and vice versa. It is also suggested that a decline in total protein content is a useful index of cellulardysfunction.Our observation is in agreement with that of Nwangwu *et al.* (2011),who documented that ethanolic leaf extract of *Vernonia amygdalina* resulted in a non-significant decrease in the total protein content of experimental animals.Several authors have reported that plant extracts prevent renal toxicity due to the presence of some secondary metabolites like tannins, flavonoids and phenolic compounds, etc. in them(Anupam and Mumtaz (2013), Sonkar et al., (2014)).Therefore the overall renal improvement potentials possessed by our combined extracts on alloxan induced diabetic rats may have been conferred on them by their high antioxidant defense capacities as reported by Atangwho *et al., (*2007),Ijeh and Ejike(2011),

The results of the liver function test are presented in table 4(U/L). There was a ratio

dependent decrease in the values of the liver enzymes, AST ALP.The values decreased as the ratio of bitter leaf extract increased. Therefore, extract ratio BI/OK|90:10 had the least values in the enzymes, AST, 43.20±2.35 and ALP,43.20±2.35.While (BI/OK|70:30) showed the highest decrease in ALT value with 10.00±0.70.However,the values were comparable to the diabetic control group. High level of these marker enzymes indicates liver and/or certain tissue cells are damaged and so compromised membrane integrity and therefore leakage of these marker enzymes into the bloodstream.

Table 4:Effect of the combined leaf extracts on liver function.

GROUP	AST (U/L)	ALT (U/L)	ALP (U/L)	T.P (g/L)	T.B (µmol/L)	CB (µmol/L)
NORMAL CONTROL	75.60±2.33^{b}	12.20±1.01^{b}	40.00±2.30^{b}	63.40±2.83^{b}	8.42±0.28^{b}	4.84±0.18^{b}
NEGATIVE CONTROL	97.80±3.61^{a}	39.00±1.14^{a}	119.00±6.46^{a}	50.40±1.86^{c}	11.59±0.29^{a}	6.78±0.18^{a}
BI/OK|10:90	74.60±2.01^{b}	26.20±1.46^{ab}	96.80±3.99^{ab}	74.20±1.95^{b}	10.44±0.25^{ab}	6.08±0.19^{c}
BI/OK|30:70	60.20±1.15^{ab}	21.60±1.60^{ab}	82.20±3.65^{ab}	70.00±1.41^{b}	10.48±0.30^{ab}	5.94±0.15^{c}
BI/OK|50:50	68.00±1.64^{b}	16.00±1.18^{b}	65.00±4.06^{ab}	83.20±3.45^{b}	9.06±0.46^{b}	5.50±0.23^{c}
BI/OK|70:30	53.60±1.50^{ab}	10.00±0.70^{b}	44.40±2.06^{b}	62.80±2.59^{c}	6.02±0.17^{ab}	4.00±0.08^{b}
BI/OK|90:10	43.20±2.35^{ab}	11.40±0.92^{b}	39.20±1.24^{b}	74.40±2.83^{b}	7.40±0.18^{b}	5.22±0.68^{b}
STANDARD DRUG (METHFORMIN)	53.00±2.50^{ab}	6.40±0.50^{ab}	34.80±4.80^{b}	80.40±1.63^{b}	7.90±0.42^{b}	4.48±0.22^{b}

Values are means ± standard deviations for 5 rats per group (n = 5)

Values in the same column with similar superscript letters are not significantly different at 5% level ($p< 0.05$).LEGEND:BI-Bitter leaf;OK-Okazi;AST- Aspartate transaminase; ALT- Alanine Transaminase; ALP- Alkaline Phosphatase;TB-Total bilirubin;CB-Conjugated bilirubin.

The serum levels of the above enzymes have been used as a pointer of not only the functionality and cellular integrity of the liver but as well, to assess the functional health status and the internal environment of the organism (Rehman *et al.,* 2006). Normally a rise in their serum levels may point to an inflammation or impairment to the hepatocytes and this happens mostly whenever the liver undergoes such pathological conditions as cirrhosis or made to pass through abnormal onslaught that accompany the presence of toxins or usage of some drugs (Crook, 2006). Wounded or inflamed liver cells release above normal amounts of certain chemicals, including liver enzymes into the blood stream thereby leading to higher concentration of the liver enzymes in the blood. The fact that the extracts had a lowering effect on the serum levels of these liver marker enzymes is a proof that it had a positive interaction on the hepatocytes. Our current findings support past observation on the hepato-protective potentials of various extracts of *V. amygdalina* in experimental animals (Ijeh and Obidoa, 2004).There was decrease in both total and conjugated bilirubin compared to the negative control. Highest decrease in both total and conjugated bilirubin was observed in Group 6 (BI/OK|70:30) with 6.02±0.17 and 4.00±0.08 respectively. However,the values for total bilirubim was not statistically different from the other groups ($P < 0.05$).While the value for conjugated was not comparable ($P < 0.05$)to other groups

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except the normal5.22±0.68and the diabetic5.22±0.68controls.Bilirubim is a byproduct of haemoglobin breakdown in the cells of the liver and spleen.When there is elevated blood bilirubin,it indicates unusual rapid breakdown of red cells or that the liver is not disposing off waste products properly.When in high concentration in the serum it piles up in some tissues leading to medical some conditions(Sedlak and Snyder, (2004)).These adverse effects of increased or elevated bilirubin in the system may have be opposed by the extract in this study, as it is seen to reduce its concentration in the blood.

Table 5 shows the results of oxidative stress marker, the result showed that there was an increase in the values of GSH compared to the negative control in all the groups although not statistically different. The group,BI/OK:90:60 had the least value, 1.20±0.02 U/L followed by the diabetic control 1.49±0.27U/L for CAT. However, their values were not statistically different from the other groups. These anti- oxidant enzymes help to confer protection or to scavenge free radicals. They are also markers of oxidative stress and low values indicate several health risks including different types of cancers. The eventual enhancement of these enzymes' activities by the extracts gives credence to the antioxidant properties possessed by combined leaf of *Vernonia amygdalina* and *Gnetum africanum* and their capabilities to enhance the enzymatic antioxidant defense mechanistic activities as reported by Iwalokun *et al*. (2006).

Table 5: **Effectof the combined leaf extracts on oxidative stress marker**.

GROUP	GSH(U/L)	CAT(U/L)
NORMAL CONTROL	1.40±0.06^{c}	2.77±0.51^{c}
NEGATIVE CONTROL	1.48±0.15^{c}	1.71±0.30^{c}
BI/OK|10:90	1.54±0.18^{c}	3.32±0.68^{c}
BI/OK |30:70	1.81±0.13^{c}	3.13±0.41^{c}
BI/OK |50:50	1.76±0.18^{c}	2.24±0.48^{c}
BI/OK |70:30	1.66±0.08^{c}	2.24±0.26^{c}
BI/OK |90:10	1.95±0.33^{c}	1.20±0.02^{c}
STANDARD DRUG (METHFORMIN)	2.47±0.17^{c}	1.49±0.27^{c}

Values are means ± standard deviations for 5 rats per group (n = 5)
Values in the same column with similar superscript letters are not significantly different at 5% level ($p< 0.05$).LEGEND:BI-Bitter leaf;OK-Okazi;GSH-Glutathione stimulating hormone;CAT-Catalase

CONCLUSION

The results from this work proved that the combined aqueous leaves extracts of *Vernonia amygdalina* and *Gnetum africanum* influence weight positively, possess hypoglycemic and anti-hyperglycemic properties, protects the integrity of both the kidney and the liver and support the antioxidant system. Therefore, the extracts could be used to manage diabetes. Moreover, since the two leaves are stable vegetables in Nigeria, they are at times eaten raw or their juice drunk and also used in different culinary preparations, their ingestion via these modes could still bestow some of these benefits on diabetics.

Conflicting interests:

There is no competing interest to declare.

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