Original Research Article

BIOCHEMICAL EFFECTS OF METHANOLIC EXTRACTS OF *VERNONIA AMYGDALINA* AND *GONGRONEMA LATIFOLIA* ON ALLOXAN-INDUCED DIABETIC RATS

ABSTRACT

Aim:The study investigated effects of combined methanol extracts of *Gongronema latifolium* and *Vernonia amygdalina* on fasting blood glucose (FBG) levels, oxidative stress markers and some haematological indices of alloxan-induced diabetic rats. **Methodology:** Twenty (25) albino wistar rats were assigned into 5 groups of 5 rats per group. Diabetes was induced in groups 2-5 by a single intraperitoneal injection of alloxan monohydrate (160 mg/kg) while group 1 rats served as normal control. Upon establishment of diabetes, group 2 rats were treated with 200 mg/kg of *G. latifolium* extract; group 3 rats with a combination of 100 mg/kg of *G. latifolium* and 100 mg/kg of *V. amygdalina*; group 4 rats with 200 mg/kg of *V. amygdalina* while group 5 rats were treated with 2 mg/kg glibenclamide. All treatments were daily through the oral route for 21 days. The FBG levels of the rats were assessed at 2 h, 6 h and on days 7, 14 and 21post-treatment while blood for clinical chemistry [Catalase, Superoxide dismutase (SOD) and Malondialdehyde (MDA)] and haematological [Red blood cell (RBC) count, packed cell volume (PCV) and Haemoglobin (Hb) concentration)] analyses were collected on day 21. **Results**: Results showed that the FBG level of the rats treated with combined extract decreased significantly ($P < 0.05$) from 203.66 ± 1.85 on day zero to 48.00 ± 3.57 on day 21. The mean catalase activity and MDA levels of the rats that received the combined treatment (group 3 rats) were statistically comparable to that of glibenclamide-treated rats. The SOD activity, RBC count, PCV levels and Hb concentration of the rats in group 3 were significantly ($P < 0.05$) higher than those of the negative control group. **Conclusion**: Treatment of diabetic rats with 100 mg/kg each of methanol extracts of *G. latifolium* and *V. amygdalina* exhibited hypoglycaemic, anti-oxidant and anti-anaemic potentials.

Key words: *Gongronema latifolium Vernonia amygdalina* , biochemical, haematology diabetic Rats,

INTRODUCTION

Diabetes mellitus is derived from the Greek word ‘diabetes’ meaning siphon (to pass through) and the Latin word ‘mellitus’ meaning honeyed or sweet. It was known in the 17^{th} century as the ‘pissing evil’ **[1].** Diabetes mellitus commonly referred to as diabetes is a group of metabolic diseases in which a person or animal has high blood sugar, either because the pancreas does not produce enough insulin, or because the body’s cells do not respond to the insulin that is being produced **[2].** This high blood sugar (hyperglycaemia) produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased appetite or hunger).

Diabetes was the cause of 4.9 million deaths in 2014 (as against 1.5 million in 2012), implying that every seven seconds, a person died from diabetes. It was also estimated that 1 in 12 people were living with diabetes including diagnosed and undiagnosed cases **[3].** In animals, diabetes is most commonly encountered in dogs and cats. Middle-aged animals are most commonly affected. Female dogs are twice as likely to be affected as males while according to some sources, male cats are also more prone than females. In both species, all breeds may be affected but some small dog breeds are particularly likely to develop diabetes such as Miniature Poodles, Dachshunds, Cairn Terriers and Beagles, but any breed can be affected **[4]**

In developing countries, few people have access to the conventional diabetes management drugs, thus, many people use plant for treatment of diabetes. Also, the inadequacies associated with the conventional medicines have led to a determined search for alternative natural therapeutic agents

Vernonia amygdalina Commonly called bitter leaf is a perennial shrub that belongs to the family *Asteraceae* and grows throughout tropical Africa **[5].** Extracts from *V. amygdalina* have been shown to posses anti-diabetic, hepato-protective, serum lipid modulation, and other properties **[6].** According to **[7]** in a study conducted on the effect of *V. amygdalina* extract on blood glucose levels of diabetic rats, there was a remarkable decrease in blood glucose level from mean value 4.44 ± 0.2 to 1.66 ± 0.2 mMol/L. Other researchers **[8, 9]** have also confirmed hypoglycemic effects of this shrub. *Gongronema latifolium* is a herbaceous, non-woody plant from the family Asclepiadaceae. It produces milky clear latex and is widespread in the tropical and subtropical regions especially in Africa and South America, with a moderate representation in Northern and South Eastern Asia **[10].** Pharmacological studies have also shown that *G. latifolium* has hypoglycemic properties **[11, 12].** In Nigeria, these two plants are used culinarily and there is a dearth of information on some biochemical effects of their combined usage.

The available synthetic drugs have complicated mode of intake and they are expensive with serious side effects. There is therefore the need to search for an alternative source of therapy thus this study. This study therefore was to investigate possible haematobiochemical changes that may be associated with the combined usage of these two shrubs on alloxan-induced diabetic rats.

MATERIALS AND METHODS

Materials

Plant Materials

The leaves of *Gongronema latifolium* (GL) and *Vernonia amygdalina* (VA) were purchased from Ogige market in Nsukka Local Government Area both in Enugu State, Nigeria and were identified by a Botanist in the Botany Department, University of Nigeria, Nsukka.

Chemical, Reagents and Drugs.

Methanol, alloxan monohydrate (SIGMA ALDRICH, U.K.), Red blood cell (RBC) and white blood cell (WBC) diluting fluids and Drabkin's reagent, Malondialdehyde (MDA), superoxide oxide dismutase (SOD) and Catalase reagents Glibenclamide (Hovid®, Hong Kong) were used

Animals

Male albino Wistar rats weighing between 150-200 g were obtained from the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka laboratory animal house. The rats were acclimatized for two weeks. The environmental temperature where the animals were housed varied between 28-32 ^{0}C. The animals were kept in stainless wire mesh cages and provided with good clean water *ad libitum*. They were fed with Vital feed® (grower).

Methods

Preparation of the Plant Extract

Cold maceration method of extraction was employed. The leaves of *G. latifolium* and *V. amygdalina* were air dried at a very low intensity of sunlight to avoid denaturation of the active ingredient. They were pulverized and stored in an air tight container pending its usage. About 2 kg of each of the pulverized leaves were soaked separately in 10 liters of 80 % methanol with intermittent shaking every 2 hours for 48 hours. The mixtures were filtered using Whatmann No 1 filter paper. The filtrates were concentrated using rotary evaporator and the extract stored at 4 ^{0}C.

Experimental Design

Twenty five (25) adult male albino wistar rats weighing between 150-200 g were assigned to 5 groups of 5 rats per group. Diabetes was induced in rats of groups 2-5 while group 1 rats served as normal control. Upon establishment of diabetes, (Rats with fasting blood glucose values above 7 mmol/L (126 mg/dl) were considered diabetic.), the rats were treated as shown below:

GROUPS	TREATMENT
1	NORMAL CONTROL + 10 ml/kg Distilled water
2	DIABETIC + 200 mg/kg GL
3	DIABETIC + 100 mg/kg GL and 100 mg/kg VA
4	DIABETIC + 200 mg/kg VA
5	DIABETIC + 2 mg/kg Glibenclamide

The treatment was through the oral route daily for 21 days. The FBG levels were assessed 2 h, 6 h, 7 days, 14 days and 21 days post treatment. On the 21st day, blood samples were collected into EDTA bottles for haematological (red blood cell, white blood cell, packed cell volume, and haemoglobin concentration) analyses while plasma was used for biochemical (superoxide dismutase, catalase and malondialdehyde) determinations.

Induction of Experimental Diabetes mellitus

Diabetes was induced in rats using the method described by **[13].** The rats were fasted for 16 h prior to induction of diabetes. Diabetes was induced by single intraperitoneal injection of alloxan monohydrate at the dose of 160 mg/kg. Diabetes was established on day two post induction on confirmation of fasting blood glucose levels above 7 mmol/l or 126 mg/dl

Estimation of Superoxide dismutase

Superoxide dismutase activity was assayed by the method of **[14].** 0.5 ml of plasma was diluted to 1.0 ml with ice cold water, followed by 2.5 ml ethanol and 1.5 ml chloroform (chilled reagent). The mixture was shaken for 60 seconds at 4°C and then centrifuged. The enzyme activity in the supernatant was determined as follows. The assay mixture contained 1.2 ml of sodium pyrophosphate buffer, 0.1 ml of PMS and 0.3 ml of NBT and approximately diluted enzyme preparation in a total volume of 3 ml. The reaction was started by the addition of 0.2 ml NADH. After incubation at 30°C for 90 seconds, the reaction was stopped by the addition of 1 ml glacial acetic acid. The reaction mixture was stirred vigorously and shaken with 4 ml n-butanol. The mixture was allowed to stand for 10 minutes, centrifuged and butanol layer was separated. The colour intensity of the chromogen in the butanol layer was measured in a spectrophotometer at 520 nm. A system devoid of enzyme served as control. One unit of enzyme activity is defined as the enzyme concentration, which gives 50%

inhibition of NBT reduction in one minute under assay conditions. SOD activity was expressed as U/ml of plasma.

Estimation of Catalase

The activity of catalase was assayed by the method of **[15].** To 0.9 ml of phosphate, 0.1 ml of plasma and 0.4 ml of H_2O_2 added. The reaction was stopped after 15, 30, 45 and 60 seconds by adding 2 ml of dichromate acetic acid mixture. The tubes were kept in a boiling water bath for 10 minutes, cooled and the colour developed was read at 530 nm. Standards in the concentration range of 20-100 µmoles was processed for the test. The activity of catalase was expressed as U/ml for plasma (U- µmoles of H_2O_2Utilised / second).

Estimation of Lipid Peroxidation (Malondialdehyde)

Lipid peroxidation was estimated by measuring spectrophotometrically, the level of the lipid peroxidation product, malondialdehyde (MDA) as described by **[16].** A volume, 0.1ml of the serum was mixed with 0.9ml of H_2O in a test tube. A volume, 0.5ml of 25% TCA (trichloroacetic acid) and 0.5ml of 1% TBA (thiobarbituric acid) in 0.3% NaOH were also added to the mixture. The mixture was boiled for 40 minutes in water-bath and then cooled in cold water. Then 0.1ml of 20% sodium dodecyl sulfate (SDS) was added to the cooled solution and mixed properly. The absorbance was taken at wavelength 532nm and 600 nm against a blank.

$$\%\ TBARS = \frac{A532 - A600 \times 100}{0.5271 \times 0.1} \quad (mg/dl)$$

Blood Collection for Haematological Analyses

Blood samples were collected from the rats using orbital technique, that is, from the retrobulbar plexus of the median canthus of the eye. Plasma for *in vivo* antioxidant assay was obtained by centrifuging the EDTA-treated blood sample and decanting the supernatant into another clean sample bottle.

Determination of Packed Cell Volume

The packed cell volume (PCV) was determined by the microhaematocrit method **[17].** Micro-capillary tubes were almost filled with the anti-coagulated blood samples and one end sealed with plasticine. The filled tubes were centrifuged at 10,000 revolutions per minute for 5 minutes using a microhaematocrit centrifuge (Hawksley, England). The PCV was read as a percentage on the microhaematocrit reader

Determination of Haemoglobin Concentration

The haemoglobin concentration (Hb) was determined by the cyanomethaemoglobin method **[18].** The blood sample (0.02 ml) was added to 5 ml of Drabkins reagent in a clean test tube. This was mixed gently and kept at room temperature for 20 minutes to react. The absorbances of both sample and standard were read, against a working reagent blank at a wavelength of 540 nm using a spectrophotometer (Lab-tech, India). The haemoglobin concentration of the blood sample was obtained by multiplying the absorbance of the sample with the factor derived from the absorbance and concentration of the standard

Erythrocyte Count

The erythrocyte count was determined by the haemocytometer method **[17].** Blood sample (0.02 ml) was added to 4 ml of red blood cell diluting fluid (sodium citrate, formaldehyde solution and distilled water) in a clean test tube, to make a 1:200 dilution. A drop of the diluted blood was charged onto the Neubaeur counting chamber and allowed to settle for 2-3 minutes. The objective (x 40) lens of the light microscope was used in carrying out the erythrocyte count, in the five groups of 16 small squares. The number of erythrocytes enumerated for each sample was multiplied by 10,000 to obtain the erythrocyte count per microlitre of blood

Total Leukocyte Count

The total leukocyte count was determined by the haemocytometer method **[17].** Blood sample (0.02 ml) of blood was added to 0.38ml of white blood cell diluting fluid (glacial acetic acid tinged with gential violet) in a clean test tube, to make a 1:20 dilution. A drop of the diluted blood was charged onto the Neubaeur chamber and allowed to settle for 2 minutes. The x10 objective lens of the light microscope was used in making a total count of white blood cells on the four corner squares. The number of cells counted for each blood sample was multiplied by 50 to obtain the total leukocyte count per microlitre of blood

STATISTICAL ANALYSIS

Data obtained from the study were analyzed using One-way Analysis of Variance (ANOVA). Duncans Multiple Range post hoc test was used to separate variant means. P (probability) values less than 0.05 were considered significant. The results were presented as mean ± Standard Error of the Mean (SEM).

RESULTS

Table 1: Effect of the Methanol Extract of *V. amydalina and G. latifolium* on the Fasting Blood Glucose (FBG) Levels of Alloxan-induced Diabetic Rats

The pre-induction fasting blood glucose (FBG) levels of the rats in groups 1-5 were statistically comparable ($P > 0.05$). However, the post-induction FBG of the rats in groups 2-5 increased significantly ($P < 0.05$) compared to the FBG of group 1 rats (normal control).

Two hours (2 h) post-treatment, the FBG of groups 3-5 rats were statistically comparable (P > 0.05) but were significantly (P < 0.05) lower than that of the group 2 rats. The FBG of the group 1 rats were equally comparable (P > 0.05) to that of the group 5 rats.

On the 6^{th} hour post treatment, the FBG levels of the rats in groups 3-5 were statistically comparable (P > 0.05) but were still significantly (P< 0.05) higher than those of the group 1 rats (normal control) and significantly (P < 0.05) lower than those of the group 2 rats.

The FBG levels of the rats in groups 2-5 were statistically comparable (P > 0.05) but were significantly (P < 0.05) higher than those of the group 1 rats on the 7^{th} day post-treatment.

On day 14 post-treatment however, the FBG levels of the rats in groups 1 & 3 were statistically comparable (P > 0.05) but were significantly (P < 0.05) higher than that of the group 5 rats. Rats in group 2 and 4 had FBG levels that were comparable (P > 0.05) to each other but were statistically higher than that of the other groups.

On the 21^{st} day post-treatment, the rats in group 3 had a significantly (P < 0.05) lower FBG level compared to other groups, the FBG levels of the rats in group 1 & 5 were statistically comparable (P > 0.05), while the FBG levels of the rats in group 2 were significantly (P < 0.05) higher than those of the rats in the other groups.

Table 2: Effect of the Methanol Extract of *Vernonia amygdalina* and *Gongronema latifolium* on Oxidative Stress Markers of Alloxan-Induced Diabetic Rats

The table indicates that the catalase activities of the rats in groups 2-5 were statistically comparable (P > 0.05) but were significantly (P < 0.05) lower than that of the group 1 rats.

The SOD activities of group 4 rats compared favorably with those of the other groups. However, the SOD activities of the rats in groups 1, 2 & 5 were significantly ($P < 0.05$) lower than those of the other groups while the SOD activities of the rats in group 3 were significantly ($P < 0.05$) higher than those of the other groups.

The MDA levels of the rats in group 5 were significantly ($P < 0.05$) lower than those of the other groups while those of the rats in group 2 were significantly ($P < 0.05$) higher than those of the other groups. However, the MDA levels of the rats in groups 1, 3 & 4 compared favorably with those of groups 2 & 5.

Table 3: Effect of the Methanol Extract of Vernonia amygdalina and Gongronema latifolium on Some Haematological Indices of Alloxanized Rats

The red blood cell (RBC) count of the rats in groups 1, 3 & 5 were statistically comparable ($P > 0.05$) while those of the rats in group 2 were significantly ($P < 0.05$) lower than those of the other groups. The RBC count of the rats in group 4 were significantly ($P < 0.05$) higher than those of the rats in group 2 but were lower than those of groups 1 & 3.

The packed cell volume (PCV) levels of the rats in group 3 compared favorably with those of the rats in the other groups. The PCV levels of the rats in group 1 was significantly ($P < 0.05$) higher than those of the other groups but was statistically comparable to ($P > 0.05$) those of group 3.

The haemoglobin (Hb) levels of the rats in groups 2-5 were statistically comparable ($P > 0.05$). The Hb levels of group 1 rats compared very well with those of group 5 rats but was significantly ($P < 0.05$) higher than those of the rats in groups 2-4.

The white blood cell counts of the rats in all the groups (groups 1-5) were statistically comparable ($P > 0.05$) to each other.

DISCUSSION

Upon the administration of alloxan monohydrate to the rats in the treatment groups (groups 2-5), there was a significant ($P < 0.05$) increase in FBG levels of the rats to levels positive for diabetes mellitus (DM) as against the normal controls. This was attributed to the effect of alloxan monohydrate. Alloxan monohydrate is a toxic glucose analogue which selectively destroys the insulin-producing β cells in the pancreas when administered to rodents and many other animal species **[19].**There was a significant ($P < 0.05$) reduction in the FBG of rats treated with *Gongronema latifolium* (GL) extract from the 1^{st} to the 21^{st} day of treatment by 51.7%. Francis *et al.,* **[12]** had earlier reported hypoglycaemic potentials of *G. latifolium*. The effect was thought to be mediated through the activation of hexokinase, phoshofructokinase, glucose-6-phosphatase dehydrogenase and the inhibition of glucose kinase in the liver **[11]**

Similarly, there was a significant ($P < 0.05$) reduction in the FBG of rats treated with *Vernonia amygdalina* (VA) extract. The percentage reduction from the initial hyperglycaemic level was 55%. The anti-hyperglycaemic effect of VA has been reported by other researchers **[8, 9].** The work of **[20]** suggests that VA may exert anti-diabetic or glucose-lowering action by a simultaneous suppression of gluconeogenesis and potentiation of glucose oxidation via the pentose phosphate pathway almost exclusively in the liver.

The decrease in FBG resulting from the treatment with glibenclamide (77.3 %) was comparable to the decrease brought about by the treatment with the combination of VA and GL extracts (76.7 %). This striking hypoglycaemic activitiy achieved by combining VA and GL could be because the phytochemical components of the different plants worked synergistically to bring about a significant ($P < 0.05$) decrease superior to either of the plants used alone.

Superoxide dismutase (SOD) is an enzyme that catalyzes the dismutation (or partitioning) of superoxide (O^-_2) radical into either ordinary molecular oxygen or hydrogen peroxide. Superoxide is produced as a byproduct of oxygen metabolism and if not regulated, causes many types of cell damage. Hydrogen peroxide is also damaging but less so and is degraded by other enzymes such as catalase. Thus, SOD is an important antioxidant defense in nearly all living cells exposed to oxygen **[21].**The study showed that rats in the group treated with a combination of GL and VA had a significantly ($P < 0.05$) higher SOD activity compared to the other groups. A synergy in the phytochemical components of both extracts is probably responsible for this.

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria, plants and animals). It catalyzes the decomposition of hydrogen peroxide to water and oxygen **[22].** It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS). Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide to less-reactive gaseous oxygen and water molecules **[23].** It has been reported that a catalase deficiency may increase the likelihood of developing type 2 diabetes mellitus **[24].** Studies have also shown that patients with diabetes mellitus usually have a reduced catalase activity **[25]**). Rats that received the combined treatment of GL and VA produced a higher catalase activity than the other treatment groups.

The Malondialdehyde levels of the rats treated with a combination of the extracts was however lower than those of the rats treated with either of the extracts signifying a less lipid peroxidative activity in this group of rats. Malondialdehyde (MDA) is the organic compound that results from the lipid peroxidation of poly unsaturated fatty acids **[26]** and it is therefore a marker of oxidative stress **[27].** This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts **[28]**

Anaemia is a common finding in patients with diabetes mellitus particularly in those with overt nephropathy**[29]**. Similarly, another study showed that the mean values of red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV) and mean corpuscular haemoglobin concentration (MCHC) for the diabetic patients were lower than the values of the control group indicating the presence of anaemia in the former group **[30].** Previous report indicates that the occurrence of anaemia in DM is due to increased non-enzymatic glycosylation of RBC membrane proteins which correlates with hyperglycaemia **[31].** On the other hand, research has shown that WBC counts are significantly higher among diabetics compared to non-diabetics and that there is a positive correlation between raised WBC levels and poor glycaemic control defined as hyperglycaemia **[32]**

The result of this study as seen in table 3 shows that rats treated with a combination of GL and VA leaf extracts had RBC counts ($6.44 \pm 0.32 \times 10^6$ millions/µl) statistically comparable to those of the normal control ($6.92 \pm 0.2 \times 10^6$ millions/µl). The RBC counts of the rats that received the combined treatment was significantly ($P < 0.05$) higher than those of the rats that received either of the extracts alone signifying a better glycaemic control as explained by Thomas and Rampersad, **(2004).**

The PCV of the rats in the treatment groups were statistically comparable but were significantly lower than those of the normal control. The rats that received the combined treatment however had PCV levels (38.66 ± 0.33 %) similar to those of the normal control (40.33

± 0.33 %). This also indicates a better glycaemic control exerted by the combination of the extracts.

The study also shows that the WBC count of all the rats in the different groups were statistically comparable although those of the rats that received the combined treatment ($6.87 \pm 0.03 \times 10^3$ thousand/µl) was closest to those of the normal controls ($6.89 \pm 0.01 \times 10^3$ thousand/µl).

CONCLUSION

The results of the present study show that the combination of the methanol extracts of GL and VA exhibited hypoglycaemic, *in vivo* anti-oxidant effects in addition to a positive effect on haematological indices superior to either of the extracts used alone.

Table 1: Effect of the Methanol Extract of *V. amydalina and G. latifolium* on the Fasting Blood Glucose (FBG) Levels of Alloxan-induced Diabetic Rats.

Group	Pre-induction FBG (mg/dl)	Post-induction FBG (mg/dl)	2 h post-treatment FBG (mg/dl)	6 h post-treatment FBG (mg/dl)	7days post-treatment FBG (mg/dl)	14days post-treatment FBG (mg/dl)	21days post-treatment FBG (mg/dl)
1	78.66 ± 0.33[a]	75.00 ± 1.15[a]	77.66 ± 0.88[a]	69.00 ± 0.57[a]	75.33 ± 0.88[a]	77.33 ± 4.48[b]	70.66 ± 1.76[b]

2	78.00 ± 2.08^{a}	209.33 ± 0.88^{c}	185.33 ± 7.53^{c}	157.66 ± 21.78^{c}	105.00 ± 2.88^{b}	104.33 ± 2.84^{c}	101.00 ± 2.08^{d}
3	78.33 ± 0.88^{a}	203.66 ± 1.85^{b}	135.00 ± 18.17^{b}	102. 00 ± 1.15^{b}	93.66 ± 4.84^{b}	68.00 ± 6.80^{b}	48. 00 ± 3.57^{a}
4	77.66 ± 4.91^{a}	204. 33 ± 1.76^{b}	116. 00 ± 7.02^{b}	120.66 ± 5.36^{b}	106.33 ± 8.76^{b}	93.66 ± 2.40^{c}	91.66 ± 2.72^{c}
5	78.66 ± 1.52^{a}	210. 00 ± 0.58^{c}	105.55 ± 3.46^{ab}	102.33 ± 1.20^{b}	102.33 ± 0.88^{b}	53.00 ± 1.00^{a}	71.60 ± 6.00^{b}

a, b, c and d indicate significant difference at $P<0.05$ down the groups (down the column).

Table 2:Effect of the Methanol Extract of *Vernonia amygdalina* and *Gongronema latifolium* on Oxidative Stress Markers of Alloxan-Induced Diabetic Rats.

Group	Catalase (U/ml)	SOD (U/ml)	MDA (g/dl)
1	5.00 ± 0.10^{b}	0.57 ± 0.03^{a}	4.39 ± 0.04^{ab}

2	2.73 ± 0.25^{a}	0.58 ± 0.05^{a}	5.88 ± 0.86^{b}
3	2.98 ± 0.26^{a}	0.74 ± 0.02^{b}	4.55 ± 0.25^{ab}
4	2.42 ± 0.62^{a}	0.64 ± 0.04^{ab}	5.45 ± 0.33^{ab}
5	3.18 ± 0.16^{a}	0.51 ± 0.04^{a}	4.18 ± 0.455^{a}

a and b indicate significant difference at $P < 0.05$ down the columns (across the groups).

SOD- Superoxide Dismutase

MDA- Malondialdehyde

Table 3: Effect of the Methanol Extract of Vernonia amygdalina and Gongronema latifolium on Some Haematological Indices of Alloxanized Rats.

Group	RBC count(x10^{6}) (millions/µl)	PCV (%)	Hb Conc. (g/dl)	WBC count(x10^{3}) (thousands/µl)
1	6.92 ± 0.20^{c}	40.33 ± 0.33^{b}	13.66 ± 0.33^{b}	6.89 ± 0.01^{a}
2	4.85 ± 0.47^{a}	37.00 ± 0.577^{a}	12.66 ± 0.16^{a}	6.66 ± 0.33^{a}
3	6.44 ± 0.32^{c}	38.66 ± 0.33^{ab}	12.83 ± 0.16^{a}	6.87 ± 0.03^{a}
4	5.64 ± 0.16^{b}	37.66 ± 0.33^{a}	12.66 ± 0.33^{a}	6.64 ± 0.22^{a}
5	6.18 ± 0.18^{bc}	38.40 ± 0.36^{a}	13.16 ± 0.16^{ab}	6.61 ± 0.31^{a}

a, b and c indicate significant difference at $P \leq 0.05$ down the columns.

RBC count- Red blood cell count

PCV- Packed cell volume

Hb- Haemoglobin

ETHICAL APPROVAL

All authors hereby declare that “principles of laboratory animal care” (NIH publication No 85-23, revised 1985) were followed, as well as specific national laws. All experimemts have been examined and approved by the appropriate ethics committee

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