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Effects of Phytobiotics on Oxidative Stress and Hematology in Streptozotocin (STZ)-induced Diabetic Wistar Rats: A Comparative Study

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Abstract The phytobiotics used in this study are turmeric (*Curcuma longa*) and bitters (extracts from leaves of *Vernonia amygdalin*). The effects on oxidative stress level and hematological parameters in streptozotocin-induced diabetic Wistar rats treated with either insulin, ethanol extracts of *Curcuma longa* (turmeric) or *Vernonia amygdalina* (bitters) was investigated for 28 days. Diabetes was induced in the animals using a single dose of streptozotocin (50 mg/kg), intraperitoneally. Confirmation of diabetes was made 48 h post induction with STZ. Thirty male Wistar rats were divided into six groups of five comprising of Group 1 (normal control), Group 2 (diabetic), Group 3 (insulin treated), Group 4 (turmeric, 400 mg/kg), Group 5 (*Curcuma longa,* 800 mg/kg), and Group 6 (bitters, 400 mg/kg). At the end of the treatment (28 days), blood was collected from the retrobulbar plexus for various biochemical analyses and animals were humanely sacrificed. Results of blood glucose revealed that at Day 0 and 7, Groups 2, 3, 4, 5 and 6 had a significantly ($p < 0.001$) high blood glucose concentration which gradually reduced and at day 28 was significantly ($p<0.001$) reduced in Groups 3, 4, 5 and 6. There were significant changes in WBC count, lymphocyte count, platelet count and granulocyte count among the groups while RBC, Hb, PCV, MCV, MCH and MCHC showed no significant difference in all the groups. The GSPx, SOD and Catalase levels in Groups 3, 4, 5 and 6 were significantly ($p<0.001$) increased when compared to Group 2 which was significantly decreased ($P<0.001$). MDA concentration was significantly increased ($P<0.001$) in Group 2 compared to normal control group, Groups 3, 4, 5 and 6. These effects were seen to be ameliorative to varying degrees in the insulin- and extract-treated groups. Turmeric and bitters have been demonstrated in this study to significantly decrease oxidative stress levels and improved altered hematological functions to appreciable levels comparable to normal control and insulin-treated diabetic groups. Specifically, *C. longa* extract (turmeric) has proved to be a potent antihyperglycemia, anti-anaemic and antioxidant extract.

Keywords: *diabetes, turmeric, Vernonia amygdalina, antioxidants, hematology, insulin, streptozotocin, Wistar rats*

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1. Introduction

Diabetes Mellitus (DM) is a multi-factoral and heterogeneous disorder with both environmental and genetic factors contributing to its development [1]. It is a chronic metabolic disorder that is estimated to affect 4% of the world's population. A doubling of this figure is expected in the near future, especially in the African and Asian continents [2].

DM is associated with long-term damage, dysfunction, and failure of various organs, especially eyes, kidneys, nerves, heart and blood vessels. Therefore, diabetes leads to reducing patients' quality of life and life expectancy. Studies have shown increased plasma triglycerides and total cholesterol typical of diabetic conditions [3] maybe a risk factor for vascular disease [4].

At moderate concentrations, free radicals including nitric oxide (NO), superoxide anion, and related reactive oxygen species (ROS) play an important role as regulatory mediators in signalling processes. Many of the ROS-mediated responses actually protect the cells against oxidative stress and re-establish "redox homeostasis" [5]. At high concentrations, however, these free radicals, radical-derived and non-radical reactive species are hazardous to living organisms and damage all major cellular constituents.

ROS is seen as the underlying cause of metabolic syndrome in obesity and can lead to damage of islets and insulin resistance leading to diabetes (). It has also been implicated in the progression of long-term diabetes complications, including microvascular and macrovascular dysfunction (). The Rotterdam study provides strong evidence that patients with T2DM are at a significantly increased risk of developing Alzheimer's disease (AD) and that the risk of AD is even higher among patients

treated with insulin [6], therefore this study will help reduce or eliminates one of the risk factors of AD.

The use of natural products is gaining popularity worldwide because they are cheap, readily available and easy to administer (). Curcumin an active component of *Curcuma longa* appears to be beneficial in preventing diabetes-induced oxidative stress in rats [7]. *Vernonia amygdalina* (bitters), through various researches have been said to have antidiabetic properties [8].

Curcuma longa commonly referred to as 'Turmeric' is a perennial rhizomatous herbaceous plant of the ginger family, Zingiberaceae. Apart from its culinary appeal and common use as a spice, it is well known for its medicinal properties in Egyptian and Indian culture for more than six thousand years. In Nigeria, the Calabar and Ibibio people call it "Adanunen" (). It is used locally on bruises, cut skin, spots; and as such rubbed on new mothers to rid their skin of whatever marks pregnancy might have left and to give their skin a health glow (). However, having learnt the other health benefits of turmeric, few urban people incorporate it into their everyday cooking as spice, besides its constituent in curry.

Curcumin, the active portion of *Curcuma longa*, has been shown to have significant antioxidant activity, both in vitro and in vivo [9]. Curcumin is said to be a potent scavenger of reactive oxygen and nitrogen species such as hydroxyl radicals and nitrogen dioxide radicals [10]. It has various other health-benefiting properties, such as hypolipidemic [11], anti-inflammatory [12], anticancer [13], antihyperglycemic and antiviral [13].

Vernonia amygdalina is a shrub that grows predominantly in tropical Africa. Leaves from this plant serve as food vegetable and culinary herb in soup [14]. It is commonly called "Bitter leaf" in English language, Onugbu in Igbo language, Etidot in Efik, Ijaw and Ibibio, Ewuro in Yoruba language, Oriwo in Edo and Chusa-doki in Hausa [15]. *Vernonia amygdalina* has been found to be rich in minerals, especially phosphorus, calcium, potassium, magnesium, zinc, iron and some vitamins like vitamins A, C and E [16]. *Vernonia amygdalina* extracts have been shown to exhibit profound ethnomedical and pharmacological properties viz, anti-diabetic [8], antimalarial [17], antihelminthic and antibiotic properties [18]. *V. amygdalina* extract possessed in vitro antioxidant activity when tested with DPPH radical scavenging assay [19].

This study therefore attempts to compare the effect of two natural edible herbs - *Curcuma longa* and *Vernonia amygdalina,* and insulin (a natural antihyperglycemic compound) on oxidative stress and haematological parameters in streptozotocin (STZ)-induced diabetic Wistar rats. This will provide baseline information and also act as a guide in treatment options with best overall outcomes for diabetes mellitus and its complications.

2. Materials and Methods

2.1. Chemicals and Reagents

The chemicals and reagents (analytical grade) used for this research included: Streptozotocin (Sigma Aldrich, St. Louis, USA), Insulin, Assay Kits (for enzyme analysis), ethanol, sodium picrate.

2.2. Equipment

The equipment used during this research included the following: refrigerator, oven, weighing balance, centrifuge, abacus 380 analyser.

2.3. Preparation of Extracts

Fresh leaves of *Vernonia amygdalina* (identification number 80b) and rhizomes of *Curcuma longa* (turmeric) (identification number 10j) were obtained from Pharmacology farm, University of Uyo, Akwa, Ibom state and authenticated by the chief curator Mrs Udoma. The leaves were rinsed severally with tap water and thereafter allowed to dry. The dried leaves were blended using a manual blender (Corona). A 368.6 g of ground leaves was extracted (cold maceration) in ethanol for 48 h. The dried rhizomes were blended (270.5 g) and extracted in ethanol by cold maceration for 48 h. The different mixtures were filtered with Chess cloth, then with Whatman No.1 filter paper separately to obtain a homogenous filtrate. The filtrates were concentrated under reduced pressure at 45°C with rotary evaporator (Büchi, Switzerland) and then to complete dryness

2.4. Acute Toxicity Test

Acute toxicity test for both extracts was carried out in Wistar rats to determine the dose levels to be administered to the experimental animals using Lorke's method [20].

2.5. Animal Handling

Thirty-six (36) male Wistar rats weighing between 200-220 g were obtained from the animal house of the Department of Pharmacology, University of Calabar, Calabar, Nigeria. Animals for the experiment were kept in accordance with ethics in the guidelines for animal care as contained in the animal ethics handbook of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria. The animals were allowed to acclimatize for 3 weeks in the animal house of the Department of Biochemistry. The animals were housed in well ventilated stainless cages and kept under controlled environmental conditions of room temperature, relative humidity and 12 h light/dark cycle and were allowed free access to standard pelletized feed (Vital Feed from Grand Cereals and Oil Mills Limited, Jos, Plateau) and water.

2.6. Experimental Procedure

2.6.1. Induction of Diabetes and Treatments

Diabetes was induced in overnight fasted animals with intraperitoneal injection of freshly prepared solution of 50 mg/kg streptozotocin in 0.1M sodium citrate buffer. The animals with fasting blood glucose >7.8 mmol/l or >180 mg/dl and above were considered diabetic and used for the study [21]. The extracts (Turmeric, CL, and bitters, VA) were reconstituted in 1 % v/v Tween20 and administered *per os*. Insulin (standard drug) was administered intraperitoneally at 5 IU/kg as described by [22]. The control animals received 0.2 ml of distilled water orally. The animals were divided into 6 groups of 5 animals each (Table 1). Treatment lasted for 28 days.

2.6.2. Experimental Design

Table 1.

Groups	Number of animals	Treatment	Dose
Normal control (1)	5	Placebo	0.2 ml distilled water
Diabetic control (2)	5	STZ (DM)	50 mg/kg
Diabetic treated (3)	5	STZ + Insulin	5 IU/kg
Diabetic treated (4)	5	STZ + turmeric	400 mg/kg
Diabetic treated (5)	5	STZ + turmeric	800 mg/kg
Diabetic treated (6)	5	STZ + *V. amygdalina*	400 mg/kg

2.7. Measurement of Fasting Blood Glucose

Fasting blood glucose (FBG) was determined at 7 days interval using glucometer (Accuchek advantage II).

2.8. Collection of Samples for Analysis

After 28 days post treatment, the animals were fasted overnight but had free access to water. Whole blood was collected from the retrobulbar plexus of the media canthus for various biochemical analyses. The blood was divided into 2 portions: 1 ml into EDTA bottles for haematological assay and the remainder emptied into plain tubes and allowed to clot. The clotted blood samples were then centrifuged at 3000 rpm for 10 min to recover serum. The animals were humanely sacrificed.

2.9. Haematological Analyses

Haematological analysis was carried out using Abacus 380 auto-analyser on whole blood.

2.9.1. Determination of Oxidative Stress Parameter and Antioxidant Markers

Assay for Superoxide Dismutase

Spectrophotometer assay for superoxide dismutase (SOD) in liver tissue was carried out using assay kit from Bioxytech ® SOD-525 ™, based on Nebot *et al*. [23].

Assay for Glutathione Peroxidase

Colourimetric assay of glutathione peroxidase (GSPx) was evaluated using assay kit from Bioxytech ® GPx-340 ™, based on Rotruck *et al.* [24].

Assay for Catalase

Spectrophotometric assay of catalase (CAT) was evaluated using assay kit from Bioxytech ® csatalase-520 ™, based on Aebi [25].

2.9.2. Quantification of Lipid Peroxidation

MDA in the sample was reacted with Thiobarbituric acid (TBA) to generate MDA-TBA adduct which was easily quantified colorimetrically at 532 nm. This assay detects MDA levels as low as 1 nmol/well calorimetrically and 0.1 nmol/well flurometrically. Lipid peroxidation (MDA) calorimetric assay kit (Biovision) was used.

2.10. Data Analysis

The results are presented as mean ± standard error of mean. Data was analyzed using a one-way ANOVA followed with a post hoc test (LSD). Data was analyzed with the aid of computer software SPSS 17 and 2016 Microsoft Excel for windows.

3. Results

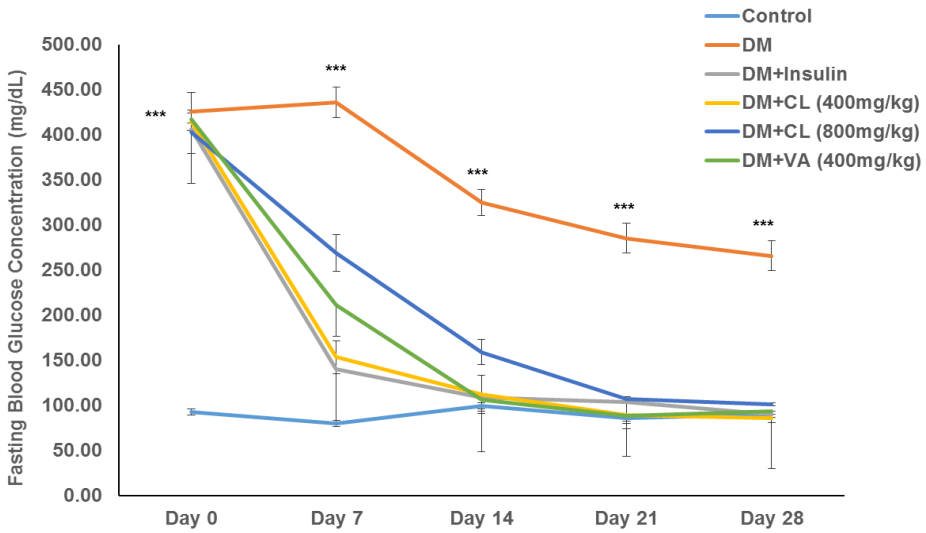
Comparison of fasting blood glucose level in the different experimental groups at days 0, 7, 14, 21 and 28.

Figure 1 shows comparison of fasting blood glucose (mg/dl) levels in Group 1 (normal control), Group 2 (diabetic control), Group 3 (DM + insulin), Group 4 (DM + CL 400 mg/kg), Group 5 (DM + CL 800 mg/kg) and Group 6 (DM + VA 400 mg/kg). The fasting blood glucose levels in groups 2, 3, 4, 5 and 6 showed a significant increase (P< 0.001) compared to group 1 (normal control). The fasting blood glucose level at day 7 in Groups 2-6 compared showed a significant increase (P<0.001) compared to control. The fasting blood glucose level at day 14 showed a significant increase (P<0.001) in fasting blood glucose level in Group 2, Group 5 and Group 6 compared to group 1. At day 21, a significant increase (P<0.001) in fasting blood glucose level was seen in group 2 compared to control. The treatment Groups 3, 4, 5 and 6 also showed a significant decrease (P<0.001) in fasting blood glucose levels when compared to Group 2 (diabetic control).

Table 2. Comparison of Haematological indices in the different experimental groups of STZ-induced diabetic rats treated with insulin, turmeric and bitters extracts

Parameters	Group 1 (Control)	Group 2 (DM)	Group 3 (DM+Insulin)	Group 4 (DM+CL 400 mg/kg)	Group 5 (DM+CL 800 mg/kg)	Group 6 (DM+VA 400mg/kg)
WBC (x10^3 cells/µL)	8.01±0.67	13.07±0.83***	10.22±0.44^{*,b}	10.16±0.61^{*,b}	10.73±0.38^{*,b}	9.22±0.25^{c}
RBC (x10^6 cells/µL)	9.17±0.33	9.16±0.37	10.12±0.60	10.43±0.41	9.11±0.87	8.71±0.55^{q}
HB (g/dL)	15.44±0.48	15.38±0.71	17.54±1.30	17.60±0.60	15.68±1.46	14.60±0.76^{q}
PCV (%)	50.30±2.08	54.54±4.38	57.71±0.62	58.18±2.14	54.22±2.27^{d}	56.89±2.00
MCV (fL)	62.00±1.05	59.40±1.54	62.00±1.10	64.60±2.34	60.20±0.75	58.00±1.45
MCH (pg)	16.84±0.30	17.22±0.12	17.80±0.23	17.30±0.47	16.80±0.46	16.86±0.12
MCHC (g/dL)	27.18±0.40	28.34±0.19	27.90±0.43	27.76±0.80	28.72±0.27	28.96±0.43
PLT (x10^{3}cells/µL)	547.40±25.06	503.40±31.88*	527.20±34.14	455.00±25.18^{***,c,e}	410.20±38.56^{***,c,e,q}	453.60±20.91^{***,c,e}
Lym (%)	4.83±0.26	10.38±0.84***	6.29±0.36^{b}	7.02±0.22^{b}	8.56±0.52*	6.37±0.14^{b}
Mono (%)	15.18±2.61	9.07±1.08***	12.24±1.44^{b}	13.46±3.47^{b}	9.14±1.04^{***,q}	9.06±1.14^{***q}
Granulocyte (%)	24.08±6.28	13.74±2.21***	25.30±2.30^{c}	18.08±3.57^{***,e}	13.44±1.82^{***,f}	17.66±3.77^{***,e}

Values are mean±SEM, n = 5. *9<0.05, ***p<0.001 vs control; b = p< 0.01 vs DM; c = P<0.001 vs DM; d =p <0.05 vs DM + insulin, e = p <0.01 vs DM + insulin, f = P < 0.001 vs DM + insulin; q = p <0.05 DM + CL, DM + VA.



Values are mean ± SEM, n=5. ***p<0.001 vs control

Figure 1. Comparison of weekly fasting blood glucose concentration between the control and the treated groups

At Day 28, a significant increase (P<0.001) in fasting blood glucose level was seen in Group 2 compared to Group 1. Groups 3, 4, 5 and 6 showed a significant decrease (P<0.001) in fasting blood glucose level compared to Group 2 and was not different from the control (Group 1).

Haematological indices in the different experimental groups.

Comparison of Total WBC count in experimental groups.

Table 2 shows that the total white blood count for the normal control, diabetic control, insulin treated, turmeric (400 mg/kg), turmeric (800 mg/kg) and Bitters. The diabetic control group showed a significant increase (P<0.00) compared to normal control. Groups 3-5 showed a significant decrease (p<0.01) compared to group 2 and a significant increase (p<0.05) compared to normal control group. Group 6 showed a significant decrease (p<0.001) compared to diabetic control group.

Comparison of differential WBC count in experimental groups.

Table 2 shows the result for lymphocytes, monocytes and granulocytes in the different experimental groups. Lymphocyte count were significantly increased in diabetic control group (P<0.001) and in turmeric (800 mg/kg) group (P<0.05) compared to normal control. The insulin group, turmeric (400 mg/kg) and Bitters groups showed no significant difference compared to the normal control group but were significantly low (p<0.01) compared to diabetic control group. The monocyte count in the diabetic, turmeric (800 mg/kg) and bitters groups were significantly lower (P<0.001) compared to control. The insulin and turmeric (400 mg/kg) Groups were significantly high (p<0.001) compared to diabetic group. Granulocyte values in diabetic, turmeric (400/800 mg/kg) and bitters groups were significantly lower (P<0.001) compared to normal control. Within the treated groups, insulin group showed a significantly increased (p<0.001) compared to diabetic group. Turmeric (400 mg/kg) and bitters Groups were significantly lower (P<0.01) while turmeric (800 mg/kg) group was significantly lower (P<0.001) compared to the insulin group.

Comparison of RBC count in the different experimental groups

The total RBC count of the experimental groups showed no statistically significant difference in RBC count among the experimental groups (Table 2).

Comparison of haemoglobin (Hb) content in the different experimental groups.

The Hb contents of the experimental groups were 15.44±0.48, 15.38±0.71, 17.54±1.30, 18.60±0.8, 15.68±1.46 and 14.60±0.76 respectively. The only statistical significance was Hb content of turmeric low dose group being higher (P<0.05) than that of control (Table 2).

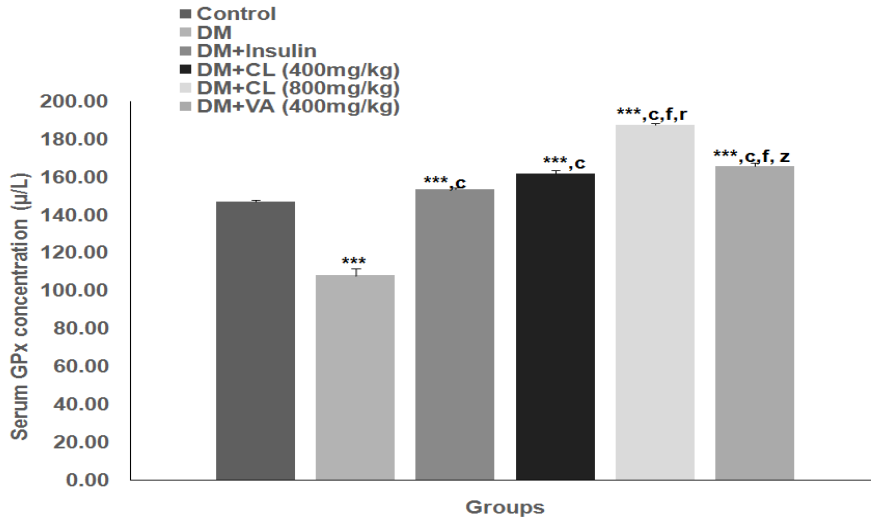
Comparison of packed cell volume (PCV) in the different experimental group.

Table 2 shows that the packed cell volume of the different experimental groups. Diabetic control and VA group showed no significant difference compared to control. The insulin treated and turmeric (400 mg/kg) group was significantly higher than control (P<0.001) and (P<0.05) respectively.

Comparison of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) in the experimental groups shows no significant difference seen in MCV, MCH and MCHC (Table 2). The values for the platelet count in the different experimental groups were 547.40±25.06, 503±31.88, 527.20±34.14, 455±25.18, 410.20±38.56 and 453.60±20.91 respectively. The diabetic group showed a significant decrease (P<0.05) compared to control. Within the treatment groups, turmeric (400/800 mg/kg) and bitters showed significant decrease in platelet count (P<0.001) compared to normal control and significant decrease (p<0.01) compared to insulin group. Bitters group also showed a significant decrease (p<0.05) compared to turmeric (400 mg/kg) (Table 2).

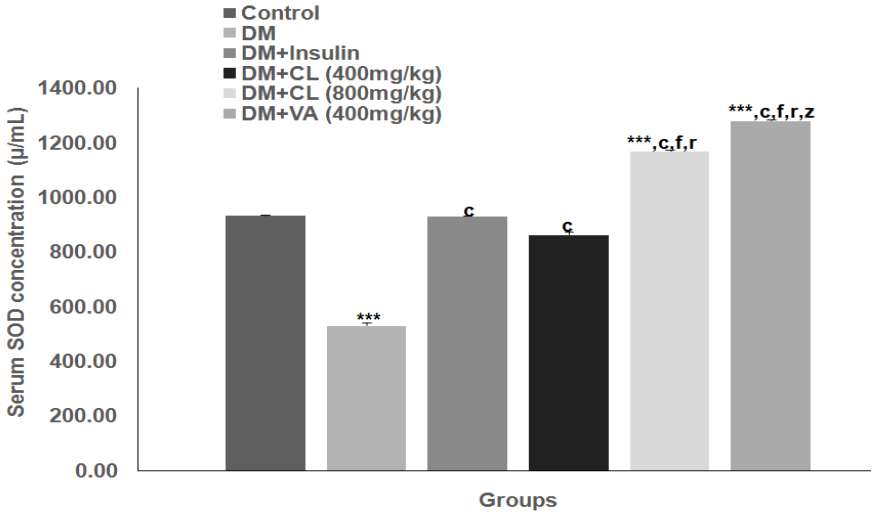
Comparison of antioxidant enzymes activities and MDA.

A comparison of antioxidant enzymes, glutathione peroxidase, superoxide dismutase and catalase levels after 28 days of treatment are shown in Figure 2, Figure 3, Figure 4 respectively.



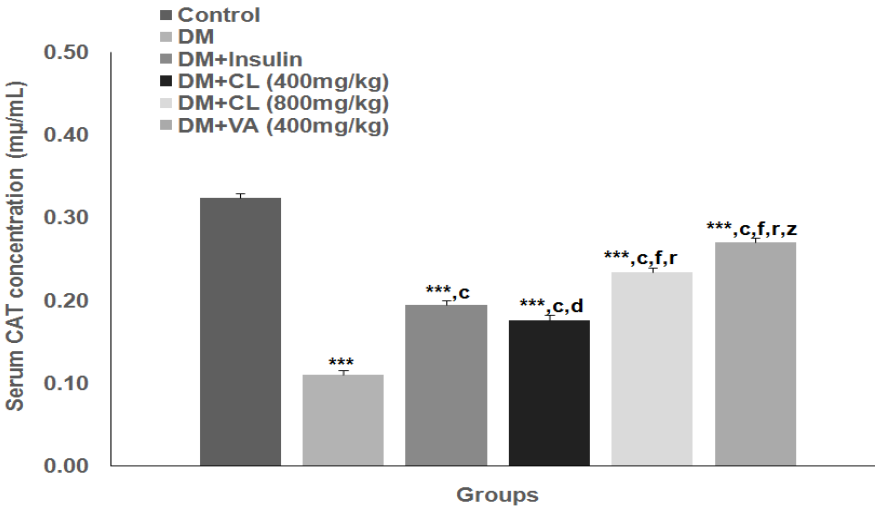
Values are mean ± SEM, n = 5. ***p<0.001 vs control; c = p<0.001 vs DM; f = p<0.001 vs DM+Insulin; r = p<0.001 vs DM+CL (400mg/kg); z = p<0.001 vs DM+CL (800mg/kg)

Figure 2. Comparison of glutathione peroxidase conc. between the control and treated groups



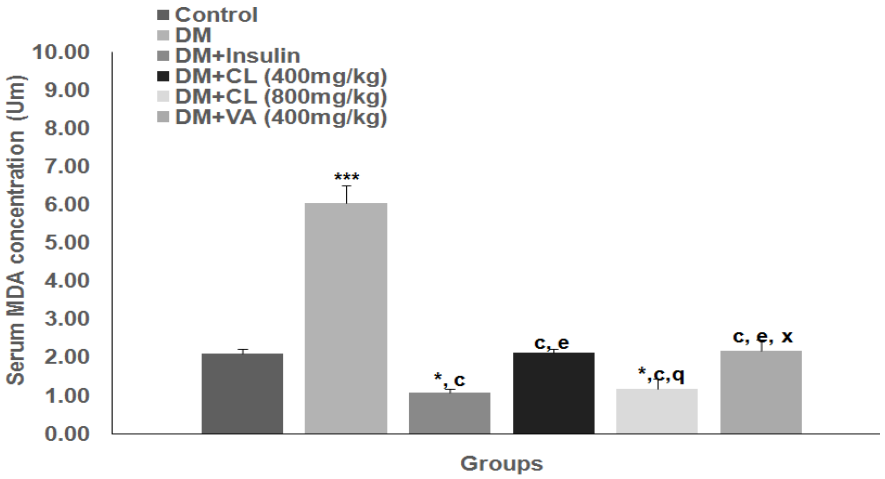
Values are mean ± SEM, n = 5. ***p<0.001 vs control; c = p<0.001 vs DM; f = p<0.001 vs DM+Insulin; r = p<0.001 vs DM+CL (400mg/kg); z = p<0.001 vs DM+CL (800mg/kg).

Figure 3. Comparison of superoxide dismutase conc. between the control and treated groups



Values are mean ± SEM, n = 5. ***p<0.001 vs control; c = p<0.001 vs DM; d = p<0.05, f = p<0.001 vs DM+Insulin; r = p<0.001 vs DM+CL (400mg/kg); z = p<0.001 vs DM+CL (800mg/kg).

Figure 4. Comparison of catalase conc. between the control and treated groups



Values are mean ± SEM, n = 5. *p<0.05, ***p<0.001 vs control; c = p<0.001 vs DM; e = p<0.01 vs DM+Insulin; q = p<0.05 vs DM+CL (400mg/kg); x = p<0.05 vs DM+CL (800mg/kg).

Figure 5. Comparison of MDA concentration between the control and treated groups

Figure 2 shows that Glutathione peroxidase (GSPx) levels in diabetic group was significantly lower (P<0.001) compared to control, insulin- and extract-treated groups.

Figure 3 shows that superoxide dismutase (SOD) levels in turmeric (800 mg/kg) and bitters group were significantly higher (P<0.001) compared to normal control. Within the treatment groups, SOD levels in turmeric (400 mg/kg) group was significantly higher (P<0.001) compared to diabetic control. turmeric (800 mg/kg) and bitters groups also showed a significant increase (P<0.001) compared to insulin and turmeric (400 mg/kg) group. Bitters group showed a significant increase (p<0.001) compared to turmeric (800 mg/kg) group.

Figure 4 shows catalase levels in all treatment groups were significantly lower (P<0.001) compared to normal control group and significantly higher (P<0.001) compared to the diabetic control group.

Figure 5 shows lipid peroxidation activities. Malondialdehyde (MDA) levels in the diabetic control group was significantly higher (P<0.001) compared to the normal control and other treatment groups.

4. Discussion

Hyperglycaemia observed in the diabetic animals as a result of administration of STZ agrees with Bonner-Weir *et al.,* that STZ induces Type 2 diabetes in many animal species at low doses and have been reported to resemble human hyperglycaemic non-ketotic diabetes [50]. This is attributed to a selective cytotoxicity on beta cells and subsequently, impaired glucose oxidation [26]. Ethanol rhizome extract of turmeric treatment in diabetic Wistar rats for 28 days effectively decreased their fasting blood glucose level and this reduction was comparable with insulin. This result confirmed earlier reports by Arun and Nalini [27] that administration of turmeric or curcumin to diabetic rats reduced blood sugar levels. Turmeric contains curcuminoids and sesquiterpenoids as well as other phytochemicals. Both curcuminoids and sesquiterpenoids exhibits hypoglycaemic effect through PPAR-γ activation [28] and PPAR-γ activation has been shown to directly improve β- cell function and survival [29]. Curcuminoids have also been reported to inhibit α- glucosidase activity therefore interfering with digestion of carbohydrates in the intestine [30].

Also, ethanol leaf extract of *Vernonia amygdalina* (bitters) also showed significant decrease in fasting blood glucose level. It has also been shown to have blood sugar lowering effect in rats [31]. Major constituents of the extract from the leaves of *Vernonia amygdalina* include sesquiterpene lactones (vernodalin, vernolide, hydroxyvernolide), and steroid glucosides (vernonioside A1-A4: for bitter tasting constituents and vernonioside B1-B3; for non-bitter related constituents) [32,33]. Suggestions have argued that the hypoglycemic effect of aloes and its bitter principle may be mediated through stimulating synthesis and/or release of insulin from the beta-cells of Langerhans [34]. *V. amygdalina* containing both sesquiterpene lactones and bitter principle may therefore act through stimulation of synthesis and/or release of insulin from the beta-cells of the pancreatic islets. Other mechanism of actions such as through the inhibition of glucose absorption, increased sensitivity of receptors to insulin, insulinase inhibiting effect and stimulation of peripheral tissues uptake of glucose are also possible [35]. Kumar *et al*. reported that a possible mechanism by which extracts exerts hypoglycaemic activities is by acting on beta cell to increase production of insulin [49].

Oxidative stress in diabetes coexists with altered antioxidant systems and a relative change in activities in diabetic tissue compared to normal usually indicates an altered antioxidant system [36]. Antioxidant enzymes glutathione peroxidase (GSPx), superoxide dismutase (SOD) and catalase (CAT) were assayed in this study. GSPx and SOD levels in the insulin- and extract-treated groups were significantly higher compared to the diabetic group, although the extract-treated groups showed a more significant increase in antioxidant enzymes compared to the insulin group. Within the extract groups, bitters-treated group showed a significant increase in SOD and catalase levels. VA was significantly lower in GSPx levels compared to *C. longa* (800 mg/kg) group. This study shows that both extracts were able to reverse antioxidant enzymes better than insulin. The antioxidant potency of *V. amygdalina* may be due to its high content of flavonoids, polyphenols

and Vitamins which are known antioxidants [37] The enzyme activity in the turmeric-treated groups increased dose dependently. Suryanarayana *et al.* [7] reported that both curcumin and turmeric resulted in considerable reversal but not complete normalization of antioxidant enzymes. Also in a study by Ogunlade *et al.* [38].

Quantification of lipid peroxidation is essential to assess oxidative stress in pathophysiological processes and conditions. Lipid peroxidation forms malondialdehyde and 4-hydroxynonenal (4-HNE), as natural by-products. MDA is a known biomarker of lipid peroxidation and oxidative stress. The increase in MDA level signifies increased toxicity. Reports by Sharma [39] showed that curcumin which is an active ingredient in turmeric was able to ameliorate renal lipid peroxidation by reducing MDA levels. Also, Owolabi *et al.* [40] reported the potency of *V. amygdalina* in attenuating oxidative damage in diabetic rabbit by lowering MDA levels. The counteraction of the extracts in reducing MDA levels shows their potency as antioxidants. Although *C. longa* (400 mg/kg) and *V. amygdalina* extracts were able to reduce the MDA levels to levels comparable in normal, the turmeric extract (800 mg/kg) was able to reduce the MDA level in dose dependent manner much better than *V. amygdalina* extract.

Anaemia is a blood disorder that is increasingly noticeable in patients with diabetes [41]. It is a clinical condition characterized by reduction in haemoglobin concentration of blood below normal levels for the age, sex and physiological problems and altitudes [42]. Studies have shown that the incidence of anaemia is mostly associated with the presence of renal insufficiency in diabetic patients because kidneys are unable to produce enough erythropoietin. Diabetic neuropathy has also been shown to affect the renal nerves, resulting in reduction in erythropoietin level, a condition that leads to anaemia [43]. In this study, RBC, MCV, MCH and MCHC levels of experimental groups treated with plant extracts showed no significant difference compared to normal control after 28 days. This therefore indicates that extract from these plants, probably owing to the bioactive components, can control diabetes-related anaemic conditions.

Turmeric (400 mg/kg) also showed an increase in PCV and HB levels compared to normal control. Studies have shown that ingestion of medicinal compounds or drugs can alter the normal range of haematological parameters [44]. Curcumin can accelerate the emulsification of fat by stimulating the production of bile [45] so that the digestion of fat will be more optimal. Optimized fat digestion will indirectly increase the provision of substrates for ß-oxidation or fat metabolism which eventually enhances the production of succinyl-CoA through Krebs metabolic cycle [46]. Considering that succinyl-CoA is one of the materials needed for haemoglobin (heme in particular) synthesis [47], enhanced succinyl-CoA production was therefore most likely to be followed by the increase of haemoglobin synthesis.

In this study, differential white blood cell (WBC) count showed lymphocyte levels of diabetic control group to increase significantly compared to normal control and also monocyte and granulocyte levels to decrease significantly compared to the normal control. The treated groups indicated a significant reduction in lymphocyte levels except for the group treated with turmeric (800 mg/kg) which was significantly still higher. The monocyte levels in insulin-treated and turmeric (400 mg/kg) showed an increase to levels comparable with normal control while the turmeric (800 mg/kg) and *V. amygdalina*-treated groups were still significantly lower compared to the normal control. The extract-treated groups apart from turmeric (800 mg/kg) showed improvement in granulocyte levels compared to diabetic untreated group. However, they were not able to reach levels comparable to the normal control. Also, the extract-treated groups showed a significant decrease in platelet counts compared to the normal control. In previous studies, turmeric has been reported to exhibit anticoagulation and antiplatelet activities [48]. Saponins which are present in a very appreciable quantity can inhibit platelet aggregation and this could be a possible explanation for the significant decrease in platelet count. This effect of turmeric may be useful for treatment of patients with vascular thrombosis or as an antithrombotic therapeutic agent in post myocardial infarction.

Turmeric and *Vernonia amygdalina* have been demonstrated in this study to significantly decrease oxidative stress levels comparable to normal control and insulin-treated diabetic Wistar rats. Specifically, turmeric (800 mg/kg) has proved to be a more potent antihyperglycemia and antioxidant compared to *Vernonia amygdalina*, with its effects comparable to insulin. Turmeric is a more potent antioxidant with ability to bring about a near total reversal of damages caused by diabetes in rats. *Vernonia amygdalina* shared these attributes but to a lesser effect. If these findings are applicable to humans, use of these phytobiotics in diabetic patients portends a promising future in the fight against diabetes and related disorders.

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