Original Research Article

Determination of Some Biochemical Parameters in Streptozotocin-Induced Diabetic Albino Rats Pre-Treated and Post-Treated with *Vernonia Amygdalina* and *Gongronema Latifolium* Extracts

ABSTRACT

The use of herbs in the management of diabetes mellitus and its complications have been reported. This study was thus aimed at determining the levels of some biochemical parameters in streptozotocin-induced diabetic albino rats pre-treated and post-treated with *Vernonia amygdalina* and *Gongronema latifolium* extracts. Fifty (50) albino rats weighing between 150 – 250g were used for this study. 25 albino rats were used for each phase of the treatment. The pre-treatment phase involved the treatment of the rats with 400mg/kg b.w (singly) and 200mg/kg b.w (combined) extracts for 14 days after which diabetes mellitus was induced using streptozotocin before the rats were sacrificed. The post-treatment phase involved the inducement of diabetes with streptozotocin after which the rats were treated with 400mg/kg b.w (singly) and 200mg/kg b.w (combined) extracts for 28days before the animals were sacrificed. Blood was collected via cardiac puncture and plasma/serum was collected. Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), malondialdehyde (MDA), and total antioxidant capacity (TAC) were determined using standard procedures while glucose was measured with a glucometer. Cardiovascular risk ratios such as Castelli risk ratio I and II and atherogenic index of plasma were also calculated. Results showed that extracts of *V. amygdalina* (only), *G. Latifolium* (only) and V.A+ GL (combined) significantly ($p<0.05$) reduced the TC, LDL-C , FBS, MDA and TAC levels while HDL-C level significantly ($p<0.05$) increased. The Cadiac risk indices (CRI-I and CRI-II, and Atherogenic index of plasma (AIP) in both the pre-treated and post-treated rats were also reduced when compared to diabetic control. It is thus evident that these plants' extracts possesses hypoglycaemic, hypolipidaemic, and antioxidant properties and thus could be used to reduce cardiovascular risks.Therefore, the tradomedicinal use of these plants in the management of cardiovsacular complications is highly recommended.

Keywords: Diabetes mellitus, Vernonia amygdalina, Gongronema latifolium, hypolipidaemic, hypoglycaemia, antioxidant, hyperglycaemia.

1.0 INTRODUCTION

A chronic hyperglycaemic condition, stemming from the decreased supply of insulin, its action or both, resulting into a cluster of metabolic disorder is termed diabetes mellitus (DM)[1]. Mainly two types of diabetes mellitus occur, such as type I and II. The former is also called the juvenile diabetes or insulin-dependent diabetes mellitus, as it occurs mainly in the young , characterized by destruction of beta cells which may be due to an autoimmune process or accident, usually leading to absolute deficiency of insulin [2]; patients with this type of diabetes will require insulin therapy to maintain normal blood glucose concentration,

Comment [M1]: in

Comment [M2]: called juvenile

while the latter is also called maturity onset diabetes or non-insulin dependent diabetes mellitus representing ninety percent of all cases of diabetes mellitus [3] , and may be due to insensitivity of target tissues to insulin [1].

Oxidative stress is a state whereby there is an imbalance between the generation and neutralization of reactive oxygen and nitrogen species (RONS) such that the antioxidant capacity of cell becomes overwhelmed [4]. When free radicals are overproduced, biomolecules such as lipids, proteins and DNA may get destroyed, leading to the induction of several disease conditions such as cancer, diabetes, cardiovascular diseases, aging and other degenerative diseases. The diabetogenic potential of streptozotocin is dependent on its ability to generate reactive oxygen species [5], which exert toxic effects on the beta cells of the pancreas, decreasing its ability to produce insulin; these free radicals may be responsible for the diabetes-induced pathological conditions. The antioxidant capacity of plasma is said to be the primary measure and marker in the evaluation of the status and potential of oxidative stress in the body. To prevent cellular biomolecules from being damaged, certain compounds occur in the plasma, and function against the oxidative stressors in the body. The sum total potential of all the antioxidant molecules in the plasma is a reflection of the antioxidant capacity of the plasma. In all processes where reduced plasma antioxidant potential is reported, prevalence of oxidative stress is also reported [6]. In diabetics with poor glycaemic control, the plasma antioxidant level is significantly low, while diabetics with good glycaemic control have higher plasma antioxidant level. Oxidative stress in diabetics coexists with a decrease in the antioxidant status, which in turn, may further elevate the deleterious effects of the free radicals.

Coronary artery disease is one of the pathologies associated with diabetes mellitus, with dyslipidemia been identified as one of the most important risk factors. Low HDL-C, high

Comment [M3]: maturity-onset

Comment [M4]: the cell

Triglyceride and high LDL-C levels have been associated with increased incidence of coronary artery disease [7]. However, the absence of an abnormal lipid profile does not completely rule out the possibility of coronary artery disease, thus high risk persons may be identified through predictions using different combinations of the lipid profile parameters; these include the Atherogenic Index of Plasma (AIP) and Castelli Risk Index (CRI) and Atherogenic Coefficient (AC) [8]. The AIP is calculated as Log TG/HDL-C, CRI-I as TC/HDL-C, CRI-II as LDL-C/HDL-C, and AC as TC– HDL-C/HDL-C [9].

The World Health Organization stated that eighty percent of the emerging world's population depends on the use of herbal medicine. Herbs being the oldest form of healthcare produce several plant extracts and phytochemicals with several therapeutic benefits and affordable treatment [10]. Plants major constituents include terpenoids, flavonoids, glycosides, alkaloids and carotenoids, which are often believed to possess antidiabetic effect.

Vernonia amygdalina (bitter leaf) is the most prominent specie belonging to the family Asteraceae [11], and mainly found in the tropical parts of Africa, [12], where it is used as a vegetable or flavour in soups. It is commonly referred to as bitter leaf due to its bitter taste in nature; the bitter taste is due to its anti -nutritional components such as alkaloids, saponins, glycosides and tannins. The leaves have found relevance in traditional folk medicine as antihelmint, a laxative herb and an antimalarial as they are known as quinine substitute [13]. It is also used in the treatment of cough and hypertension [14,15]

Gongronema latifolium (utazi) is a nutritional and medicinal plant which is edible, and commonly found in Nigeria, particularly in the rain forest zones, and the tropics in other African countries. As a result of their nutritional and ethnomedicinal values, several studies have been reported regarding methanolic and ethanolic extracts of the herbs. *Gongronema latifolium* (Asclepiadaceae) is also a tropical rainforest plant primarily used as spice and

Comment [M5]: with an increased

Comment [M6]: species

Comment [M7]: antihelminth

vegetable in traditional folk medicine [16,17]. Reports by various authors showed that it contains essential oils, saponins and pregnans among others [18,19. The leaves of *Gongronema latifolium* have protective role against diabetes, hypertension, stomach upsets and pains, and typhoid fever [20].

Most studies conducted on the herbs focussed mostly on the efficacy of the extracts following induction of organ damage. Literature on the prophylactic efficacy of the aqueous extracts of these herbs before induction of organ damage to evaluate the therapeutic potentials of the herbs in protecting the body organs against xenobiotic assaults are scarce. Thus, this study was designed to determine the serum levels of some biochemical parameters in streptozotocin-induced diabetic albino rats pre-treated and post treated with *Vernonia amygdalina* and *Gongronema latifolium*.

Comment [M8]: The literature

2. MATERIALS AND METHODS

This study was carried out at the Animal House, Department of Physiology, University of Port Harcourt, Nigeria. Fifty (50) albino rats weighing between 150 – 250g were used for this study. They were allowed to acclimatize a week prior to experimentation. They were kept in properly ventilated cages, at a room temperature of about 27^0C and 12 hour light/dark cycle, and the animals were fed with growers' marsh and water obtained from tap *ad libitum*.

Comment [M9]: hours

Fresh mature leaves of *V. amygdalina* and *G. Latifolium* were purchased from the Mile 1 market, Port Harcourt, Nigeria. Botanical identification was confirmed at the Herbarium, Department of Plant Science and Biotechnology, University of Port Harcourt Rivers State. The leaves were washed and air-dried in a shade for 4 days, and the dried leaves were milled into powder with a blender and stored in an airtight plastic bag and kept from sunlight. 1g of the powdered herb was soaked in 100ml of distilled water for 24 hrs and filtered with a

Comment [M10]: in the shade

muslin cloth to produce a concentration of 10mg/ml of the aqueous extract used for the experiment.

In other to induce diabetes mellitus, the adult albino rats were allowed to fast over night, and were then injected with a single intraperitoneal dose of streptozotocin at 50 mg/kg b.w in 0.1 molar citrate buffer, pH 4.5, while the control animals were injected intraperitoneally with citrate buffer alone (1 ml/kg b.w). All animals were allowed free access to feed and water after streptozotocin (STZ) injection, and they were left undisturbed for a minimum of 72 hours for hyperglycaemia to develop. Thereafter, fasting blood glucose levels of the animals were measured with One Touch Ultra Mini Glucometer. Animals with blood glucose greater than or equal to13.8 mmol/l were considered hyperglycaemic.

Comment [M11]: afterthat

During the experimental period, there was strict adherence to ethical regulations required for handling experimental animal in accordance with National and Institutional Guidelines for Protection of Animal Welfare [21].

There were two phases; phase I (the pre-treatment phase) which was for 14 days, and phase II (the post-treatment phase) which was for 28 days, and the dosage of the plant extracts was 400 mg/kg, administered twice daily using the method of Atangwho *et al*. [22]. In phase I (pre-treatment phase), the animals were divided into five (5) groups with 5 animals in each group.

Group A (Normal Control): consisted of rats which were maintained on food (Grower's marsh) regime and water 0.1 M. citrate buffer

Group B (Diabetic Control): consisted of rats injected with streptozotocin at 50 mg/kg b.w in 0.1 M. citrate buffer, and maintained on food (Grower's marsh) regime and water

Group C: consisted of rats pre–treated with 400mg/kg of *Vernonia amygdalina* twice daily, and food (Grower's marsh) regime and water prior to induction with streptozotocin

Group D: consisted of rats pre-treated with 400mg/kg of *Gongronema latifolium* twice daily, and food (Grower's marsh) regime and water prior to induction with streptozotocin.

Group E: consisted of rats pre-treated with combined extracts of *Vernonia amygdalina* (200mg/kg) and *Gongronema latifolium* (200mg/kg) twice daily, and food (Grower's marsh) regime and water prior to induction with streptozotocin.

The rats in groups C, D and E after being pre-treated with the various extracts for 14 days, were then allowed to fast overnight on day 15, before injecting with streptozotocin (50 mg/kg), after which they were left for 72 hours, and were then sacrificed.

In phase II (post-treatment phase), the animals were divided into five (5) groups with 5 animals in each group.

Group A (Normal Control): consisted of rats which were maintained on food (Grower's marsh) regime and water 0.1 M. citrate buffer

Group B (Diabetic Controls): consisted of rats injected with streptozotocin at 50 mg/kg b.w in 0.1 M. citrate buffer, and maintained on food (Grower's marsh) regime and water

Group C: consisted of diabetic rats treated with 400mg/kg of *Vernonia amygdalina* twice daily post diabetic induction with food (Grower's marsh) regime and water.

Group D: consisted of diabetic rats treated with 400mg/kg of *Gongronema latifolium* twice daily post diabetic induction with food (Grower's marsh) regime and water.

Group E**:** consisted of diabetic rats treated with combined extracts of 200mg/kg each of *Vernonia amygdalina* and *Gongronema latifolium* twice daily post diabetic induction with food (Grower's marsh) regime and water.

The rats in groups C, D and E after successful diabetic induction, were treated with the various extracts for 28 days. After the last dose on day 28th, the animals were left fasting overnight and sacrificed on the morning of day 29.

About 6 mls of whole blood was obtained through cardiac thoracic puncture using a sterile syringe and needle; 3 mls was poured into an EDTA and 3 mls into a plain tube (allowed for some minutes to clot). Samples were spun for 10 minutes at 4000 rpm to obtain plasma and serum. Serum samples were used for the analysis of total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C), while plasma samples were used for total antioxidant capacity (TAC) and malondialdehyde (MDA) levels.

Comment [M12]: 6ml

Comment [M13]: 3 ml

Comment [M14]: 3 ml

2.1 DETERMINATION OF SERUM TOTAL CHOLESTEROL

The enzymatic procedure for total cholesterol determination in serum based upon the Trinder [23] method as modified by the Centers for Disease Control and Prevention was used. The method is popularly known as the enzymatic endpoint method. The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

2.2. Determination Of High-Density Cholesterol

The method of Lopes-Virella *et al.* [24) for the determination of high-density cholesterol in serum was employed. Low density lipoproteins and very low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoprotein) fraction, which remains in the supernatant, is determined by CHOD-PAP method.

The low density lipoprotein cholesterol (LDL-C) was calculated using the formula below:

LDL–C (mg/dl) = Total cholesterol + (HDL –Cholesterol + Triglyceride/5) [25].

The Castelli's Risk Index I and II were calculated from the formulas below:

Castelli's Risk Index (CRI)-I = TC/HDL-C

Castelli's Risk Index (CRI)-II = LDL-C/HDL-C

The Atherogenic Index of Plasma (AIP) is calculated as Log TG/HDL-C [9]

2.3. Determination of Serum Triglycerides (Tg)

Comment [M15]: TG

The colorimetric method of Tietz [26] was employed. The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4-amino phenazone and 4-chlorophenol under the catalytic influence of peroxidase.

2.4. Estimation of MDA Levels

Malondialdehyde (MDA), a marker of Lipid peroxidation was determined as thiobarbituric acid reactive substance according to Okhawa *et al.* [27] with slight modification by Atawodi *et al.* [28] using trichloroacetic acid (TCA) and thiobarbituric acid. The product of the reaction is a coloured complex which absorbs light at 533nm and can hence be measured. Exactly 2 ml of 15% trichloroacetic acid was measured into a test tube, 2 ml thiobarbituric acid was added and 100ul of the serum was added. The mixture was incubated at 80ºC for 30minutes in a water and allowed to cool for some time followed by centrifugation at 3000 rpm for 10 minutes. A clear supernatant was collected and the absorbance of it was determined at 533nm spectrophotometrically. TBARS concentrations were expressed as µmol/ml/mg of protein.

Conc. µmol/ml/mg Protein = Absorbance of sample / 1.56 x 10^{-5} x protein Conc. (mg).

2.5. Estimation of Total Antioxidant Activity

Total antioxidant activity was determined according to the method described by Buico *et al*. [29]. Preformed radical monocation of 2,2-azinobis-(3 ethyl benzothiazoline 6 sulfonic acid) (ABTS), a blue green chromophore was generated by reacting 7M ABTS stock solution with 2.45M potassium persulfate solution in acetate buffer (PH 4.5). The solution was kept in the dark at room temperature for 12 to 24 hours before use. The ABTS solution was diluted to an absorbance of 1.00 at 734 nm. 50µl of sample was added to 950µl of diluted ABTS. The sample was properly mixed and incubated in the dark in a water bath at 37^0C for 20minutes. The absorbance was read at 734nm. Trolox was used for the calibration of the method. Inhibition of absorbance level versus trolox concentration curve was used to express the serum/plasma TAC in trolox equivalent

Comment [M16]: T

2.6. Statiscal analysis

Values obtained were presented as mean ± standard error of mean (SEM). The statistical tool used was the one way analysis of variance (ANOVA) followed by the Tukey's multiple comparison tests using the IBM SPSS Version 23 Software. Results were considered statistically significant at 95% confidence interval ($p<0.05$).

3.0 RESULTS

The table 1 shows that the MDA levels in the albino rats pre-treated and post-treated with the combined extracts of *V. Amydalina* and *G. latifolium* were significantly ($p<0.05$) reduced when compared with the response obtained for the single administration of the extracts. level The TAC level, however, were reduced significantly ($p<0.05$) in the rats that were post treated for 28 days after induction of diabetes mellitus in all the groups. The pre-treatment of

223 the rats with the extracts for 14 days whether alone or in combination showed poor glycaemic
224 response while significantly reduced level of fasting blood sugar was obtained in the 28 days
225 post-treated rats after induction of diabetes mellitus.

226

Table 1. Comparison of mean ± SD of MDA, TAC and FBS levels in the pre-treated and post treated albino rats.

Groups	MDA (nmol/L) (14 days)	MDA(nmol/L) (28 days)	TAC (mmolFe/L) (14 days)	TAC (mmolFe/l) (28 days)	FBS (mmol/l) 14 (days)	FBS (mmol/l) 28 days
Group A (N.C)	92.07 ±9.90[a]	101.35 ± 7.35[b]	3.65 ± 0.19[a]	3.33 ± 0.39[a]	4.06 ± 0.024[a]	4.78 ± 0.32[a]
Group B (D.C)	214.81 ± 21.19[b]	220 ± 6.85[a]	9.65 ± 1.19[b]	12.94 ± 0.71[b]	19.44 ± 1.87[b]	21 ± 2.10[b]
Group C (*V. amygdalina*)	191.41 ± 20.37[d]	223.46 ± 13.63[c]	8.85 ± 0.97[c]	5.42 ± 1.23[c]	16.8 ± 0.99[c]	6.08 ± 0.84[c]
Group D (*G. latifoluim*)	217.58 27.31[b]	158.64 ± 35.61[d]	10.01 ± 1.24[b]	5.52 ± 0.49[c]	15.5 ± 1.29[c]	5.1 ± 0.84[e]
Group E (combined extract)	184.27 ± 23.47[c]	189.17 ± 5.67[e]	10.85 ± 1.22[e]	4.15 ± 0.10[d]	23.3 ± 1.55[d]	6.2 ± 0.68[c]
F value	4.405	8.038	11.872	32.501	54.840	37.669
P value	0.002*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

Notes: Groups with different superscript are significantly different at p<0.05, *significant at p<0.05, MDA=malondialdehyde, TAC=total antioxidant capacity, FBS= fasting blood sugar

The effect of pre-treatment and post treatment of the albino rats with the extracts on lipid profiles is shown in table 2. The table shows that while no significant ($p>0.05$) reduction in the total cholesterol and triglycerides levels in the groups were observed in the pre-treated rats before induction of diabetes mellitus, the values of total cholesterol and triglycerides were significantly (p,0.05) reduced with all the extracts. However, while the combined extracts showed more potential in causing significant ($p<0.05$) increase in the HDL-C level inrats pre-treated for 14 days, *G. latifolium* demonstrated the highest rise in HDL-C levels after 28 days of post treatment. The LDL-C level was also significantly reduced in the rats that were administered the extracts in both phases.

The CRI-I and CRI-II obtained in the albino rats pre-treated with the extacts as shown in table 3 was lowest in the animals that recieved the combined extracts of *G. latifolium* and *V. Amygdalina* and *G. Latifolium* respectively while the AIP was lowest in the rats that recieved *V. Amygdalina* and indices were far lower than that obtained from the diabetic control group B.

The comparison of the atherogenic potentials of the extracts is shown in table 3. *G. Latifolium, V. Amygdalina* and the combined extracts showed obvious potentials in reducing atherogenic risk based on CRI-I, CRI-II and AIP in the rats in the both phases of the study.

Table 2. Comparison of mean ±SD of lipid profile in rats pre-treated and post-treated with the extracts.

Groups	TC (mmol/l) 14 days	TC (mmol/l) 28 days	TG (mmol/l) 14 days	TG (mmol/l) 28 days	HDL-C (mmol/l) 14 days	HDL(mmol/l) 28 days	LDL-C (mmol/l) 14 days	LDL (mmol/l) 28 days
Group A (N.C)	1.72 ± 0.2^{a}	1.88 ± 0.05^{a}	0.62 ± 0.18^{a}	0.5 ± 0.06^{a}	0.92 ± 0.24^{a}	1.16 ± 0.04^{a}	0.51 ± 0.22^{a}	0.47 ± 0.04^{a}
Group B (D.C)	1.78 ± 0.07^{a}	1.97 ± 0.07^{a}	0.78 ± 0.17^{a}	0.9 ± 0.20^{b}	0.65 ± 0.32^{b}	0.69 ± 0.05^{b}	0.77 ± 0.37^{b}	0.86 ± 0.05^{b}
Group C (*V. amygdalina*)	1.87 ± 0.18^{a}	1.47 ± 0.19^{b}	0.84 ± 0.42^{a}	0.5 ± 0.07^{a}	0.87 ± 0.19^{c}	0.75 ± 0.10^{b}	0.69 ± 0.22^{c}	0.49 ± 0.10^{a}
Group D (*G. latifoluim*)	1.76 ± 0.18^{a}	1.57 ± 0.19^{b}	0.70 ± 0.3^{a}	0.64 ± 0.16^{a}	0.96 ± 0.09^{a}	0.83 ± 0.60^{c}	0.44 ± 0.07^{d}	0.45 ± 0.11^{a}
Group E (combined extract)	1.98 ± 0.34^{a}	1.48 ± 0.13^{b}	0.63 ± 0.17^{a}	0.37 ± 0.38^{c}	1.11 ± 0.14^{d}	0.75 ± 0.29^{b}	0.59 ± 0.19^{e}	0.5 ± 0.14^{a}
F value	2.285	2.881	1.396	5.881	5.763	7.555	2.475	3.506
P value	0.052*	0.005	0.241*	0.003^{*}	<0.001*	0.001^{*}	0.038*	0.025^{*}

Note**:** Groups with the different superscript are significantly different from each at p<0.05, *significant at <0.05, TC=total cholesterol, HDL-C=high density lipoprotein cholesterol, LDL-C=low density lipoprotein cholesterol, TG= triglycerides.

Table 3. Comparison of mean ±SD of atherogenic indices in rats pre-treated and post-treatedwith the extracts.

Groups	CRI-I (14 days)	CRI-I (28 days)	CRI-II (14 days)	CRI-II (28 days)	AIP (14 days)	AIP (28 days)
Group A (N.C)	1.95±0.19	1.64 ± 0.08	0.62 ±0.17	0.41±0.05	-0.17 ± 0.08	-0.37±0.05

Group B (D.C)	3.60±1.08	2.88 ± 0.17	1.91±0.91	1.27 ± 0.12	0.12 ± 0.11	0.12 ±0.03
Group C (*V. amygdalina*)	2.21 ± 0.16	1.99 ± 0.13	0.82 ± 0.11	0.68 ± 0.12	-0.05 ± 0.06	-0.17 ±0.04
Group D (*G. latifoluim*)	1.84±0.11	1.88 ± 0.13	0.47± 0.05	0.55 ± 0.13	-0.17 ± 0.09	- 0.15 ± 0.06
Group E (combined extract)	1.79 ± 0.1	1.97 ± 0.13	0.53 ±0.08	0.66 ± 0.16	-0.25 ± 0.03	-0.31 ± 0.042

Note: : CRI- I= Castelli Risk Index I, CRI-II= Castelli Risk Index II, AIP= Atherogenic Index of Plasma

4.0 DISCUSSION

The results from this study showed that in both phases of the study, injection of the rats with streptozotocin (diabetic control group) induced a significant increase in the blood sugar level when compared with the normal control group. This observation is in agreement with that of Akpaso *et al.* [30]. The diabetogenic potential of streptozotocin is dependent on its ability to generate reactive oxygen species [5], which exert toxic effects on the beta cells of the pancreas, decreasing its ability to produce insulin. The hypoglycaemic potential of the *V. amygdalina* and *G. latifolium* was observed to be more pronounced in rats that recieved the extacts after induction of diabetes mellitus by streptozotocin. This observation is in agreement with the reports of Uchenna *et al.* [31] and Owu *et al.* [32] who reported that the leaves of the plants possesses anti-diabetic potential in that it reduces blood glucose levels in streptozotocin-induced diabetic rats.

The mean plamsa malondialdehyde level for the diabetic control was observed to be significantly ($p<0.05$) higher than that of the normal control in both phases of the study. This may be a resultant effect of lipid peroxidation, which may be attributed to hyperglycaemia-induced oxidative stress. This report agrees with that of Akpan and Usoh [33] who stated that rats treated with streptozotocin induced a significant increase in the levels of malondiadehyde. The report by Szkudelski [5] that streptozotocin induces increased generation of reactive oxygen species also agrees with the observation in this study. Pretreatment of the rats with combination of *V. amygdalina* and *G. amygdalina* extracts showed lower significant ($p<0.05$) reduction in the malondialdehyde level. However, single administration of *G. amygdalina* for 28 days after streptozotocin-induced diabetes mellitus also showed very remakable decrease in malondialdehyde level. Nwanjo *et al.* [34] had

Comment [M17]: plasma

Comment [M18]: malondialdehyde

Comment [M19]: pre-

Comment [M20]: remarkable

earlier reported that *V. amygdalina* extract has the potential to reduce malondiaaldehyde levels in rats.

Total antioxidant capacity (TAC) is the measure of the amount of free radicals scavenged by a test solution [35], and it is used to evaluate the antioxidant capacity of biological samples [36]. Total antioxidant capacity (TAC) includes both enzymatic antioxidants, such as catalase, and non-enzymatic antioxidants, such as ascorbic acid [37]. Studies on total antioxidant capacity in albino rats administered with V. *amygdalina* and G. *latifolium* extracts following diabetic induction is scarce. The total antioxidant capacity rats pretreated with the extracts of V. *amygdalina* and G. *latifolium* were significantly higher when compared to that seen the animals post-treated with the herbs after iabetic induction. This observation could suggest that pretreatment with the plants could have the potential to protect the body from cellular damage than when it is used therapeutically after the damage has been done. The increase in toatal antioxidant capacity in the body has been reported to be due to defensive mechanism by the body's antioxidant system in response to the increased oxidative stress [38].

The result of the study further showed that the total cholesterol concentration obtained in the rats pretreated with either single extract of V. *amygdalina* and G. *latifolium* or combination of V. *amygdalina* and G. *latifolium* were not significantly different from the concentration in the diabetic control group. However, in the rats post-treated for 28 days after streptozotocin diabetic induction, the total cholesterol level was significantly ($p<0.05$) reduced when compared to the diabetic control group. The reduction in total cholesterol concentration was not significantly ($p<0.05$) different between V. *amygdalina* and G. *latifolium* aqueos extracts at the dose of 40mg/kg b.w. of the rats. Ugwu *et al.* [39] had reported that diets preparations made with V. *amygdalina* and G. *latifolium* decreased the serum total cholesterol levels,

Comment [M21]: check spelling

Comment [M22]: diabetic

Comment [M23]: pre-

Comment [M24]: total

however, their observation that *Vernonia amygdalina* diet induced a significantly lower ($p<0.05$) serum total cholesterol when compared to the *Gongronema latifolium* diet preparation was not made in this study. The triglycerides levels in the rats that recieved the combined extracts of V. *amygdalina* and G. *latifolium* in the pretreated rats were insignificantly ($p>0.05$) reduced when compared to the diabetic control rats . The study also showed that the triglycerides level in rats given the combined extracts for 28 days after induction of diabetes was significantly ($p<0.05$) reduced when compared to the levels in the groups that recieved the extracts singly. Agwu *et al*. [39] also reported the lowering of the serum triacylglycerols by the two diet preparations V. *amygdalina* and G. *latifolium* was not significant to each other at equal concentration when compared. The result is in line with the results obtained by Nwanjo [40] and Ugochukwu *et al*. [17]. The results suggest that the plants could reduce hepatic triacylglycerols biosynthesis and favor the redistribution of cholesterol among the lipoprotein molecules. Adaramoye *et al*.[41] observed no significant difference in plasma triacylglycerol levels of rats fed on *Telfairia occidentalis* supplemented diets when compared to cholesterol-fed rats. The combined extract also resulted in a significantly reduced triglycerides level in the 28 days post induction rats when compared to the levels from the plants singly.

The result of this study also that treatment aqueous extract of *Gongronema latifolium* and *Vernonia amygdalina* resulted in a significant ($p<0.05$) increase in the level of HDL-C when compared with the diabetic control group. The increase in HDL-C was more pronounced in the rats that were pretreated with extracts for 14 days before diabetic induction. Furthermore, the increase was more evident in rats that were pretreated with the combined extracts for 14 days. Since HDL-C is often regarded as the good cholesterol, this study thus shows that these plants have potential protective role against cardiovascular disease (CVD). The comparison of the effects of these plants in increasing the levels of HDL-C in serum in this study showed

Comment [M25]: received

that *G. latifolium* induces a significantly higher HDL-C concentration than *V. amygdalina*. This finding is in sharp contrast with the finding of Agwu *et al*. [39] the fact that *Vernonia amygdalina* induced a significantly higher HDL-C concentration compared to *Gongronema latifolium*. There was a non-significant decrease in the mean HDL level between the diabetic and the normal control groups.

In this study, the effects of pretreatment for 14 days and post treatment for 28 days of aqueous extracts of *Gongronema latifolium* and *Vernonia amygdalina* singly and in combination on the serum LDL-C were also compared. The results show that both preparations significantly lowered the serum LDL-C values though the *Gongronema latifolium* preparation produced a significantly lower serum LDL-C concentration relative to the *Vernonia amygdalina* extracts in both phases of treatment. LDL-C is associated with CVD because they transport cholesterol to the arteries which could lead to the formation of plaque. Therefore, plasma LDL-C level may be used for monitoring the treatment of patients with elevated cholesterol levels. From the results obtained, the plants elicited beneficial effects by lowering the serum LDL in rats.

Evidence from the present study confirms the effects of aqueous extracts of *Gongronema latifolium* and *Vernonia amygdalina* preparations on lipid levels in experimental animals. *Gongronema latifolium* and *Vernonia amygdalina* in single preparations and in combinations were found to be very effective in reducing the levels of serum cholesterol, triacylglycerols and LDL-C thereby exhibiting hypocholestrolaemic effects. They also increased the levels of serum HDL-C in the experimental animals.

Estimation of cardiovascular risk has become the cornerstone of cardiovascular prevention. Although atherogenesis is a multifactorial process, abnormalities in lipoprotein metabolism are one of the key factors, representing around 50% of the population-attributable risk of

developing cardiovascular disease [42]. The total/high-density lipoprotein (HDL) cholesterol ratio, known as the atherogenic or Castelli risk index I and or the LDL/HDL cholesterol ratio also known as Castelli risk index II are two important components and indicators of vascular risk, the predictive value of which is greater than the isolated parameters. In this respect, an increase in total cholesterol concentration, and specifically LDL cholesterol, is an atherogenic lipid marker, whereas reduced HDL cholesterol concentration is correlated with numerous risk factors, including the components of the metabolic syndrome, and probably involves independent risk [43]. The Castelli Risk indices (CR1-I) obtained from the animals following pre-treatment the extracts before induction of diabetes mellitus showed that G. *latifolium* reduced the atherogenic effect of diabetes better than V. *amygdalina* while the combined extract was better effect than both of them. Similar CRI-I findings were observed from 28 days post treatment with the extracts after induction of diabetes except that the CRI-I for the animals treated with the combined extracts was higher than that obtained from the 14 days pretreatment. The CRI-II obtained from the study followed the same trend as the CRI-I for both the 14 days preatment and the 28 days post treatment. Thus, V. *amygdalina* and G. *latifolium* exhited the capacity to reduced cardiovscular risk.

Comment [M26]: spelling

Many clinical studies make effort to introduce a better marker of atherogenic dyslipidemia that can predict the risk of CVD to be useful for evaluating response to treatment instead of the classical ratio [44]. It has been shown that Atherogenic Index of Plasma (AIP) is a strong marker to predict the risk of atherosclerosis and coronary heart disease [45,46]. AIP is calculated according to the formula, log(TG/HDL-C) [46]. The extracts of V. *amygdalina* and G. *latifolium* either in combination or singly reasonably caused a reduction of the AIP in the experimental animals in both phases of the study implying that these plants can reasonably be employed in the treatment of cardiovascular and coronary heart disease. This finding is the

first to be reported about the medicinal value of V. *amygdalina* and G. *latifolium* respectively.

5.0 CONCLUSION

The hypoglycaemic, hypolipidaemic and antioxidant properties of V. *amygdalina* and G. *latifolium* has been demonstrated this study implying that aqueous extracts of the plants can be useful as prophylactive preventive therapy in conditions of dyslipidaemia, cardiovascular disease and coronary heart disease. The novel discovery that the plants have immense potential to reduce cardiovascular risk ratios such as total/high-density lipoprotein (HDL) cholesterol ratio, known as the atherogenic or Castelli risk index I (CRI-I), LDL/HDL cholesterol ratio also known as Castelli risk index II (CRI-II) and Atherogenic index of Plasma (AIP), calculated according to the formula, log(TG/HDL-C) has added immensely to the numerous ethnopharmacological usefulness of V. *amygdalina* and G. *latifolium* in the management of cardiovascular disease and associated conditions.

Comment [M27]: prophylactic

CONFLICT OF INTEREST: None

REFERENCES

1. Ozuogwu JC, Obimba KC, Belonwu CD, Unakalamba CB. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J Physio Pathophysio. 2013; 4(4): 46-57.
2. Kumar PJ, Clark M. Textbook of Clinical Medicine. Pub: Saunders, London, UK. 2002; 1099-1121.

3. Gonzalez E, Johansson S, Wallander M, Rodriguez L. Trends in the prevalence and incidence of diabetes in the UK: 1996-2005. J Epidem Comm Health. 2009; 63: 332-336.
4. Sellamuthu PS, Arulselvan P, Kamalraj S, Fakurazi S, M. Kandasamy M 2013. Protective nature of mangiferin on oxidative stress and antioxidant status in tissues of streptozotocin-induced diabetic rats. ISRN Pharmacol.2013; 10.1155/2013/750109
5. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physio Res Acad Scient Bohemoslovaca. 2001;50: 537–46
6. Schiffrini EL. Antioxidants in hypertension and cardiovascular disease. Mol Interv. 2010; 10(6):354-362.
7. Fein R, Scheuer J. Heart Disease i Diabetes Mellitus: Theory and Practice. In: Rifkin. H. & Porte, D. Eds. Elservier, New York.1990; 812-823.
8. Pinto X, Munoz A, Prat J, Masana L. Lipoproteinratios: physiological significance and clinical usefulness in cardiovascular prevention. Vasc Health Risk Manag. 5:757-765.
9. Bhardwaj S, Bhattacharjee J, Bhatnagar MK, Tyagi S. Atherogenic index of plasma, castelli risk index and atherogenic coefficient- new parameters in assessing cardiovascular risk. Res Art Biol Sci. 2013; 3 (3): 359-364
10. Adikw E, Bokolo B. Ethanolic leaf extract of Ocimum gratissimum ameliorates methotrexate-induced nephrotoxicity in albino rats. Asian J Med Sci. 2018; 9 (5): 37-43.
11. Johri RK, Singh C. Medicinal uses of Vernonia species. J Med Aromatic Plant Sci. 1997;19: 744–752
12. Erasto P, Grierson DS, Afolayan AJ. Antioxidant constituents in *Vernonia amygdalina* leaves. Pharmaceut Bio. 2007;45(3): 195-199
13. Farombi EO. African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agents. Afr. J. Biotech. 2003; 2: 602- 671
14. Kambizi L, Afolayan AJ. An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. J. Ethnopharmacol. 2001; 77(1):5-9.
15. Amira CA, Okubadejo NU. Frequency of complementary and alternative medicine utilization in hypertensive patients attending an urban tertiary care in Nigeria. BMC Compl. Altern. Med. 2007;7: 30-48.
16. Ugochukwu NH, Babady NE. Antioxidant effects of *Gongronema latifolium* in hepatocytes insulin dependent diabetes mellitus. Filoterapia. 2002; 73 (7-8): 612-618.
17. Ugochukwu NH, Babady NE, Cobourne M, Gasset SR. (2003). The effect of *Gongronema latifolium* extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. J Biol Sci. 2003; 28 (1): 1-5.
18. Morebise O, Fafuso MA, Makinde JM, Olajide OA, Awe EO. Antiinflammatory property of Gongrenema latifolium, Phytother Res. 2002; 16:s75-s77
19. Schneider C, Rotscheidt K, Breitmaimeri E. Four new pregnane glycosides from *Gongronema latifolium* (Asclpidaeceae). Annalen Der Chemie. 1993;10:1057-1062.
20. Etim OE, Akpan EJ, Usoh IF. Hepatoxicity of carbon tetrachloride: protective effect of *Gongronema latifolium*. Pak. J. Pharm. Sci. 2008;21:268-274.
21. Public Health Service. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Publication of the Department of Health and Human Services. National Institute of Health . Office of Laboratory Animal Welfare. 2015

22. Atangwho IJ, Ebong PE, Eyong EU, Williams IO, Eteng MU. Comparative Chemical composition of leaves of some antidiabetic medicinal plants: *Azadirachta indica*, *Vernonia amygdalina* and *Gongronema latifolium*. Afr J Biotec. 2009; 8: 4685-4689.
23. Trinder P. The principle of assay for enzymatic determination of cholesterol. Ann Clin Biochem. 1969; 6:24.
24. Lopez–Virella MF, Stone P, Eltis S, Colwell JA. (1977). Cholesterol determination in HDL separated by three different methods. Clin Chem. 1977; 23**:** 822-884.
25. Friedewald WT, Levy RI, Fredrickson DS. (1972) Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 1972; 18:499-502
26. Tietz NW. Clinical Guide to Laboratory Tests. 3nd edition, Philadelphia, W.B. Saunders Company, PhiladelphiaUSA. 1995; 1-178.
27. Okhawa H, Ohisi N, Yagi K. Assay of lipid peroxide in animal tissues by thiobarbituric acid reaction. Ann Biochem. 1979; 95: 351-358.
28. Atawodi SE, Adekunle OO, Bala I. Antioxidant, organ protective and ameliorative properties of methanol extract of anogeissus leiocarpus stem bark against carbon tetrachloride-induced liver injury. Inter J Pharmaceut Sci Res. 2011; 2(6): 1443-1448.
29. Buico A, Cassino C, Ravera M, Betta P, Osella D. Oxidative stress and total antioxidant capacity in human plasma. Redox Report. 2009; 14(3): 125-131.
30. Akpaso MI, Atangwho IJ, Akpantah A, Fisher VA, Igiri A, Ebong E. Effect of Combined Leaf Extracts of *Vernonia amygdalina* (Biter leaf) and Gongronema latifolium (utazi) on the Pancreatic B-cells of Streptozotocin. Br J Med Med Res. 2011; 1 (1): 24-34.
31. Uchenna VO, Chinwe EO, John MO, Ijeoma OE. Hypoglycaemic indices of *Vernonia amygdalina* on postprandial blood glucose concentration of healthy humans. Afri J Biotec. 2008; 7: 4581-4585.
32. Owu DD, Nwokocha CR, Obembe AO, Essien AD, Ikpi DE, Osim EE. Effect of *Gongronema latifolium* ethanol leaf extract on gastric acid secretion and cytoprotection in streptozotocin-induced diabetic rats. West Ind Med J. 2012; 61(9): 852-860
33. Usoh IF, Akpan HD (2015). Antioxidative Efficacy of Combined Leaves Extracts of *Gongronema Latifolium* and *Ocimum Gratissimum* on Streptozotocin-Induced diabetic rat models. Int. Inv. J. Med. Med. Sci. 2015; 2(6): 88-95
34. Nwanjo HU, Okafor MC, Oze GO. Anti-lipd peroxidative activity of *Gongronema latifolium* in Streptozotocin-induced diabetic rats. Nig J Physio Sci. 2006; 21(1-2): 6165.
35. Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Rad Bio Med. 2000; 29: 1106–1114
36. Marques SS, Magalhães LM, Tóth IV, Segundo MA. Insights on antioxidant assays for biological samples based on the reduction of copper complexesthe importance of analytical conditions. Inter J Mol Sci. 2014;' 15:11387–1402.
37. Gupta S, Choi A, YU HY, Czerniak, SM, Holick, EA, Paolella, LJ, Agarwal AA, Combelles CMH. Fluctuations in total antioxidant capacity, catalase activity, and hydrogen peroxide levels of follicular fluid during bovine folliculogenesis. Reprod Fert Dev. 2011; 23(5): 673-680.doi:10.1071/RD10270
38. Mortazavi SB, Khajehnasiri F, Allameh A, Akhondzadeh S, Hashemi H. (2013). Total Antioxidant Capacity and Mallondialdehyde in Depressive Rotational Shift Workers. J Env Pub Health. 2013; 150693

39. Ugwu CE, Alumana EO, Ezeanyika LUS. Comparative effect of the leaves of *Gongronema latifolium* and *Vernonia amygdalina* incorporated diet on the lipid profile of rats. Biokemistri. 2009; 21: 59-65.
40. Nwanjo, H. U. Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat models. Nig J Physio Sci. 2005; 20: 39-42.
41. Adaramoye OA, Achem J, Akintayo OO, Fafunso MA. (2007) Hypolipidemic effect of *Telfairia occidentalis* (Fluted pumpkin) in rats fed a cholesterol rich diet. J Med Food. 2007; 10: 330-336.
42. Yusuf, Hawken S, Öunpuu S (2004). Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet. 2004; 364: 937–952.
43. Ascaso J, González SP, Hernández MA. Management of dyslipidemia in the metabolic syndrome. Recommendations of the Spanish HDL. Form Amer J Cardiovas Drugs. 2007;7: 39–58.
44. Mudhaffar SK. Atherogenic Index of Plasma (AIP) As a Parameter in Predicting Cardiovascular Risk in Males Compared To the Conventional Dyslipidemic Indices (Cholesterol Ratios). Karbala J Med. 2013; 6(1):1506-1513
45. Nwagha UI, Ikepeazu EJ, Ejezie FE, Neboh EE, Maduka IC. Atheogenic index of plasma as useful predictor of cardiovascular risk among postmenopausal women in Enugu, Nigeria. Afri Health Sci. 2010; 10(3): 248-252
46. Dobiasova M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apo B-lipoprotein-depleted plasma. (FERHDL). Clin Biochem. 2001; 34:583- 88.