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ANTIDIABETIC POTENTIALS OF COMBINED AQUEOUS EXTRACTS OF *GONGRONEMA LATIFOLIUM* AND *OCIMUM GRATISSIMUM* ON ALLOXAN-INDUCED DIABETIC *RATTUS NOVERGICUS*

Eyo, J. E*, Chukwu, J. N.

Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State, Nigeria.

*joseph.eyo@unn.edu.ng

Abstract

The effect of combined aqueous leaves extracts of *Ocimum gratissimum and Gongronema latifolium* on alloxan-induced diabetic *Rattus norvegicus* was investigated for 28 days. Lethal dose (LD_{50}) and phytochemical compositions were determined by standard methods. Phytochemical analyses to determine the levels of alkaloid, flavonoid, tannin and saponin content of *O. gratissimum and G. latifolium* were done using standard methods. A total of 90 rats were divided into six groups (1 – 6) of fifteen rats per group, with each group comprising of three replicates of five rats each. Diabetes was induced in the rats by injection of 150 mg/kg of crystalline powdered alloxan monohydrate. Treatment groups 2, 3 and 4 had diabetic rats administered 100, 250 and 350 mg/kg of combined extracts respectively. Group 1(normal control, non-diabetic rats), 5 (standard control, diabetic rats) and 6 (negative control, diabetic rats) were administered distilled water, standard drug (glibenclamide) and distilled water respectively. All the administration was done by oral intubation. Changes in body weight blood glucose levels, triglycerides, high density lipoprotein cholesterol and low density lipoprotein cholesterol were determined using standard methods on days 0, 7, 14, 21 and 28. The lethal dose (LD_{50}) of the mixed extract was estimated to be ≥ 5000 mg/kg of body weight of rat. The aqueous extract of *O. gratissimum* had high presence of reducing sugars, tannin, steroids, alkaloids, flavonoids, and phenol. Soluble carbohydrates, hydrogen cyanide, terpenoid and saponins were slightly present. The aqueous extract of *G. latifolium contains* tannin and alkaloids in proportionate abundance, followed by reducing sugars, soluble carbohydrates, flavonoids and phenol. A non-significant decrease ($p>0.05$) was recorded in the body weight of rats. Significant decreases ($p<0.05$) were recorded in the blood glucose levels of rats. There were no significant differences ($p>0.05$) in the lipid profiles. The result therefore, suggests that *G. latifolium* and *O. gratissimum* have hypoglycaemic, effects on alloxan–induced diabetic rats. Generally, they are non-toxic even at high dose of 350 mg/kg body weight.

Keywords: Antidiabetic potentials, Combined aqueous extracts, *Gongronema latifolium, Ocimum gratissimum* Alloxan-induced diabetes, *Rattus novergicus,* Body weight, Blood glucose levels, Triglycerides, High density lipoprotein cholesterol, Low density lipoprotein cholesterol

Introduction

Diabetes mellitus is a prevalent disease affecting the citizens of both developed and developing countries. It is estimated that 25 % of the world population is affected by this disease [1]. It is a chronic disease characterized by elevated blood glucose levels and disturbances in carbohydrate, fat and protein metabolism. Diabetes mellitus results when pancreatic beta cells are unable to maintain adequate insulin secretion to prevent hyperglycaemia. It is observed as the body's inability to effectively regulate the sugar balance which leads to severe complications such as hyperglycaemia, obesity, neuropathy, nephropathy, retinopathy, cardiopathy, osteoporosis and coma leading to death. Pancreatic damage resulting in the dysfunction of α and β cells causes disordered glucose homeostasis. In diabetic individuals, the regulation of glucose levels by insulin is defective, either due to defective insulin production which is called insulin-dependent diabetes mellitus (IDDM, Type 1) or due to insulin resistance that is termed non insulin-dependent diabetes mellitus (NIDDM, Type 2) [2]. Type 1 diabetes usually begins in childhood and is thought to be a result of autoimmune destruction of the pancreatic beta cells (the cells that produce insulin; also called islet cells). Destruction of the beta cells results in a complete loss of insulin production, thereby necessitating insulin injections to maintain blood sugar control. Type 2 diabetes is usually diagnosed after 40 years of age. Type 2 diabetes is frequently associated with insulin resistance and normal or even elevated levels of insulin, although subnormal insulin levels are also seen in some type 2 diabetics. Gestational diabetes is characterized by hyperglycemia during pregnancy and usually disappears after the child is delivered. However, even though gestational diabetes may be relatively short-lived, it can compromise the health of both mother and foetus. Diabetes is associated with a number of significant medical problems. Severe hyperglycemia may result in coma or even death. Milder hyperglycemia, if present for many years, increases the risk of cardiovascular disease, which can manifest as a heart attack, congestive heart failure, stroke, gangrene of the extremities (necessitating amputation in some cases), or kidney failure [3]. The normal range for blood glucose level is between 70 – 110 mg/dl. Insulin is a hormone that helps to maintain normal blood glucose level by making the body's cell absorbs glucose (sugar) so that it can be a source of energy. In people with diabetes, glucose levels build up in the blood and urine causing excessive urination, thirst, hunger and problems with fats and protein metabolism because the body cannot convert glucose into glycogen and fat, it begins to break down stored fats for fuel. This produces increasing amounts of acidic compounds in the blood called ketone bodies which interfere with cellular respiration energy producing process in cells. The lesions in the pathophysiology of diabetes are multiple and therefore would require more than a single drug agent to reverse all or majority of the aspects of the disease. The effective therapeutic approach should be multimodal and in this light, several traditional medicinal herbs have been preferred given the plethora of active ingredients present in a single herb [4]. Medicinal plants have formed the basis of health care throughout the world since the earliest days of humanity and have remained relevant in both developing and the developed nations of the world for various chemotherapeutic purposes. The use of plant derived natural compounds as part of herbal preparations for alternate source of medication continues to play major roles in chemotherapy especially in third world countries. Several studies carried out have shown that traditional medicines could provide better glycaemic control than currently used conventional drugs [5, 6]. Plants by means of secondary metabolism contain a variety of herbal and non-herbal ingredients that can ameliorate disease condition by acting on a variety of targets in the host organism. On the basis of the above, polyherbal therapy is considered the preferred therapeutic approach to management of diabetes mellitus given its multi-factorial pathogenicity [7].

Polyherbal therapy, which is the use of a combination of various agents from different plant sources for therapeutic purposes is a current pharmacological principle and has the advantage of producing maximum therapeutic efficacy with minimum side effects [7]. This enhanced efficacy is thought to derive from phytochemicals endowed traditional medicinal plants, since they present exciting opportunities for the development of new types of therapeutics for the management of diabetes mellitus. Such phytochemicals include tea polyphenols which suppress post-prandial hyperglycaemia and glucose transport across the small intestine [8] and saponins which delay glucose transfer from the stomach to the small intestine [9, 10]. Epicatechin has a restorative effect on pancreatic β-cells against alloxan damage [11], and plant flavonoids which exert their antidiabetic activity via antioxidant properties [12]. These reports have accelerated the global efforts to harness and

harvest those medicinal plants that bear substantial amount of potential phytochemicals showing multiple beneficial effects in combating diabetes and diabetes related complications. In the laboratory, the antidiabetic activities of *Azadirachta indica* (AI), *Vernonia amygdalina* (VA) and *Gongronema latifolium (*GL) have been reported. In a recent report, the chemical components thought to exert the antidiabetic action were compared [4]. Although extracts from these plants have individually demonstrated antidiabetic action, recent evidence from the laboratory showed that antidiabetic efficacy of the extracts was enhanced when given in combination [7, 13]. Accordingly, the present study was designed to investigate the effect of combined aqueous extracts of *Ocimum gratissimum* and *Gongronema latifolium on* alloxan-induced diabetic *Rattus novergicus*. Several studies have recommended the antidiabetic efficacies of both *Ocimum gratissimum* and *Gongronema latifolium.* The combined antidiabetic potentials of these leaves have not been reported; which is the goal of this work since these leaves are always available and affordable. The objectives of the study were to determine the effect of the combined leaf extracts on the body weight, and elucidate the effects of the extracts on blood sugar level and lipid profile of diabetic rats.

Material and Methods

Plant materials

The *O*. *gratissimum* and *G*. *latifolium used* for the experiment were bought from Obollo-Afor market in Nsukka, Nigeria. The plants were identified to species level at the Herbarium unit of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka using the method of Gbile [14] where voucher specimens were kept.

Preparation of plant material

The method of Akah *et al.* [15] was used for extraction of the aqueous plant extracts. Fresh leaves each of *O. gratissium* and *G. latifolium (*2000 g) were washed, cut into small pieces, allowed to dry to a constant weight and homogenized in a warring blender. The resulting mixture was soaked in 2 litres of distilled water. The mixture was allowed to stand 24 hours, with intermittent shaking. Following filtration, the filtrate was evaporated to dryness over a water bath and the weight of the crude extract determined [15]. The extract was kept in refrigerator (4° C) thereafter; the extract was later reconstituted in normal saline (0.85 % NaCl) at a concentration of 1 g/ml before administration on each day of the experiment.

Animal model

Ninety (90) male adult white Wistar strain albino rats weighing 200 to 250 grams, bred in the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka was used for the study. They were fed *ad labitum* with 18 % crude protein (Guinea feed) commercial broiler growers mash. They were allowed to acclimatize under standard photoperiodic condition in a clean rat cage in the Postgraduate Research Laboratory, Department of Zoology Environmental Biology University of Nigeria, Nsukka. The animals were maintained under standard laboratory condition for temperature (26 ± 2^{o} C), humidity and light. They were allowed free access to food and water.

Induction of diabetes mellitus

The method of Osinubi *et al.* [16] was used to induce diabetes in the rats. Two grams of crystalline powdered alloxan monohydrate was dissolved in 50 ml of normal saline to yield a concentration of 40 mg/ml. 150 mg/kg body weight of alloxan was administered intraperitoneally to the rats after overnight fast (access to only water was allowed) of twelve hours to make them more susceptible to developing diabetes. Only rats with serum glucose levels between (250 – 400 mg/dl) after two weeks were considered diabetic for the experiment.

Phytochemical analysis

The alkaloid, flavonoid, tannin and saponin content of *Ocimum gratissimum* and *Gongronema latifolium* were determined using standard methods [17 – 20].

Alkaloid determination

This was done according to Okwu and Omodamiro [17]. Five grams (5 g) of pulverized *O. gratissimum* and *G. latifolium* samples was weighted into a 250 ml beaker and 200 ml 10 % acetic acid in ethanol added and covered to stand for four hours. This was filtered while the filtrate was concentrated in a water bath to one-fourth of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until precipitation is complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide solution and then filtered. The residue was weighted and noted as the total alkaloid.

Determination of total phenols

This was done by the spectrophotometric method as described by Obadoni and Ochuko [18].

A fat-free sample of *O. gratissimum* and *G. latifolium* was boiled with 50 ml of ether for 15 minutes. Five grams of the extract was measured into a 50 ml flask and then 10 ml of distilled water was added. The sample was made up to the 50 ml mark and left to react for 30 minute for colour development. The absorbance of the colour was red in a spectrophotometer at 505 nm [18].

Determination of tannin content

This was determined by the method of Van-Burden and Robinson [19]. Five hundred grams of the sample was weighed into a 100 ml plastic bottle and 50 ml of distilled water added and shaken for 2 hours in volumetric flask and made up to the 100 ml mark. Then 5 ml of the filtrate was pipette into a tube and mixed with 3 ml of 0.1M $FeCl_2$ in 0.1 M HCl and 0.008M potassium ferricyanide. The absorbance of the colour developed was red at 120 nm within 10 minutes. A standard was prepared by using tannin acid.

Saponin determination

This was determined using the method of Obadoni and Ochuko [18]. Two grams of the ground product of *O. gratissimum* and *G. latifolium* was added to 200 ml of 20 % ethanol. The suspension formed was heated for 4 hours with continuous stirring at 55 ºC. The mixture was filtered and the residue re-extract with another 200 ml of 20 % ethanol. The resulting extract was reduced to about 20 ml in a water bath at 90^o C. The concentrate was transferred to a 250 ml separating funnel and 10 ml of diethyl ether added. The aqueous layer was recovered, while the ether layer was discarded. The purification process was repeated by adding 30 ml of n-butanol. The combined n-butanol extract was washed two times with 5 ml of 5 % aqueous sodium chloride. The remnant solution was evaporated in a water bath. The sample was dried in the over to a constant weight. The saponin content was calculated in percentage [18].

Flavonoid determination

Flavonoid content was determined according to the method of Boham and Kocipla [20]. Ten grams of pulverized product of *O. gratissimum* and *G. latifolium* was dissolved in aqueous methanol at room temperature. The resulting solution was filtered using Whatman filter paper. The filtrate was later be transferred into a crucible and evaporated to dryness over a water bath and weighted to a content weight.

Lethal dose (LD_{50}) determination

The lethal dose (LD_{50}) of the extract was determined according to the method of Lorke [21]. A preliminary test was done using three graded doses of the combined extract (10, 100 and 1000 mg/kg). Each dose served as a group with three mice each. No deaths were recorded after 24 hours, another three higher doses (1600, 2900 and 5000 mg/kg of body weight) were used as groups with two mice each. The number of animals dead after 24 hours was recorded. A probit curve was then plotted from which the LD_{50} was deduced.

Experimental design

A total of 90 male rats were used for the experiment. The rats were assigned to six (6) different groups (1 – 6) of 15 animals each using the completely randomized block design. Each group was divided into 3 replicates of 5 animals each. Group 1 served as the normal control and was administered distilled water by oral administration. Groups 2 – 4 were given graded doses (mg : mg) of *Ocimum gratissimum* and *Gongronema latifolium* mixed extract at 150, 250 and 350 mg/kg respectively. These doses were safe sub-lethal dosages derived from the lethal dose (LD_{50}). Groups 5 and 6 served as the drug control (administered 5 mg of glibenclamide i.e. diabetic and treated) and diabetic control (diabetic and untreated), respectively. Baseline studies were done before the commencement of extracts and drug administrations. Blood samples were collected every seven days for 28 days.

Lipid analysis

Total cholesterol

Total plasma cholesterol was determined according to the method described by Kishi *et al.* [22] using commercially available kit. Cholesterol esters are hydrolyzed by cholesterol esterase into free cholesterol and fatty acids. Free cholesterol is oxidized to cholest-4-en-3-on and hydrogen peroxide (H_2O_2). Hydrogen peroxide in the presence of phenol and amino-4-antipyrin forms a complex of red colour that is absorbed at 500 – 550 nm. The tubes was shaken and incubated for 5 minutes at 37^o C. Absorbance was read in a spectrophotometer at 550 nm.

Determination of blood glucose concentration

Fasting blood glucose was determined using a commercially available kit glucometer (Acucheck).

Lipoprotein assay

High density lipoprotein cholesterol

High density lipoprotein cholesterol (HDL-C) was first of all be precipitated in the presence of phosphotungistic acid and magnesium chloride. Five hundred µl of plasma sample was added into marked Eppendorf tubes. Then 50 µl of precipitating reagent was pipette into the same tube. After mixing, the tube was centrifuged at 10,000 rpm for 15 minutes. The clear supernatant was pipetted into a tube and the absorbance was read at 550 nm [23].

Low density lipoprotein cholesterol

Low density lipoprotein concentration was calculated using the method of Friedwald *et al.* [23] as: LDL (mg/dl) = total cholesterol – HDL + (total triglyceride ÷ 5).

Triacylglycerol

Total triglyceride was determined by the enzymatic method described by Buccolo and David [24] using a commercially available kit. Triglycerides were hydrolyzed to glycerol and free fatty acids. Glycerol was phosphorylated with adenosine-5-triphosphate (ATP) to form glycerol-1-phosphate (G-1-P) and adenosine-5-diphosphate (ADP) in the reaction catalyzed by glycerol kinase. The triglyceride working reagent was prepared according to the manufacturer's instructions while the spectrophotometer will be set at 550 nm.

Statistical analysis

Results obtained were recorded as mean ± SEM. One way analysis of Variance (ANOVA) was used to determine significant differences between treatment and control means (α = 0.5). Duncan's Least Square Difference was used to determine differences between treatment means [25].

Results

Phytochemical compositions of the aqueous leaves extracts of Ocimum gratissimum and Gongronema latifolia

Results of the qualitative phytochemical analysis revealed that the aqueous extract of *Ocimum gratissimum* had the highest presence of reducing sugars, followed by tannin, steroids, alkaloids, flavonoids, and phenol. Soluble carbohydrates, hydrogen cyanide, terpenoid, and saponins were slightly present. On the other hand, the aqueous extract of *Gongronema latifolia* contains tannin and alkaloids in proportionate abundance, followed by reducing sugars, soluble carbohydrates, flavonoids, and phenol. Hydrogen cyanide, terpenoids, and saponins were slightly present (Table 1). Quantitatively, significant variations were recorded in the reducing sugar, tannin, soluble carbohydrate, hydrogen cyanide, alkaloids, glycosides, terpenoid and phenol contents of the aqueous leaves extracts of *O. gratissimum* and *G. latifolia* (Table 1).

Lethal dose (LD_{50}) of the mixed aqueous leaves extracts of Ocimum gratissimum and Gongronema latifolia

The lethal dose test conducted with graded doses of the mixed extracts of the plant materials used in this study recorded no mortality (Table 2). As a result, the lethal dose (LD_{50}) of the mixed extract was estimated to be ≥ 5000 mg/kg of body weight.

Effects of the aqueous extracts of the combined plant extracts on the body weight of rats

There were no significant variations in the body weight of rats in all experimental groups on day 0. On day 7, rats treated with 250 and 350 mg/kg of the mixed extract had significantly lower body weights when compared with the normal control rats ($p<0.05$), but were similar to the drug-treated rats. Similar fluctuations in the mean body weights were recorded on days 14 and 21 except that on day 21 rats given 350 mg/kg of the mixed extract had significantly higher body weights compared to the drug-treated rats ($p<0.05$) (Table 3). By day 28, rats administered 250 mg/kg of the mixed extract had significantly lower body weights when compared to the normal rats, whereas rats administered 250 and 350 mg/kg of the mixed extract had significantly higher body weights compared to the drug-treated rats ($p<0.05$) (Table 3). Duration-wise, the mean changes body weights recorded in all the experimental groups on day 7, 14, 21 were significantly lower than the day 0 values ($p<0.05$). Compared to day 0 the mean body weights on day 28 were similar though exact values were not restored (Table 3).

Effects of the aqueous extracts of the combined plant extracts on the blood glucose concentration (mg/dl)

On day 0 of the experiment (Table 4), all the rats treated with graded doses of the mixed aqueous extract of *O. gratissimum* and *G. latifolia* had significantly higher blood glucose concentration when compared with the normal control rats ($p<0.05$). Also, the standard drug-treated and diabetic untreated controls had significantly higher serum glucose concentration when compared to the

normal rats. Subsequently (days 7, 14, 21 and 28), rats treated with 150 mg/kg of the mixed extract and those treated with the standard drug had significantly higher blood glucose concentration when compared with the normal control rats. Compared to the standard drug-treated rats, rats administered 250 and 350 mg/kg of the mixed extract had significantly lower blood glucose concentration ($p<0.05$). Similar dose-dependent trends were recorded in all the treatments (mixed extracts and standard drug) in which significant reductions in the blood glucose concentrations occurred on all experimental days compared to the day 0 values ($p<0.05$).

Effects of the aqueous extracts of the combined plant extracts on the total cholesterol concentration (mg/dl)

Changes in serum cholesterol concentration (CHOL) of rats treated with varying doses of the mixed extracts did not vary significantly when compared to all the controls from day 0 to 28 of the experiment ($p>0.05$) (Table 5). Insignificant time-dependent changes in the total cholesterol concentrations occurred in all the experimental groups on day 28 when compared to the day 0 values ($p>0.05$).

Effects of the aqueous extracts of the combined plant extracts on the triglyceride concentration (mg/dl)

On day 0, significantly higher serum triglyceride (TAG) concentrations were recorded in rats administered 150 and 350 mg/kg of the mixed extract when compared to the diabetic, untreated control. Comparatively, the TAG concentrations of the extract-treated rats were not significantly different from the normal and drug controls. By day 7 (Table 6), variations in the TAG concentrations of the mixed extract-treated rats were insignificant compared to the normal and drug controls. On day 14, rats orally administered 250 mg/kg of the mixed extract had significantly higher HDL concentrations than the normal control rats; others were insignificant ($p>0.05$). On day 21, rats administered 250 mg/kg of the extracts had significantly lower triglyceride concentration when compared with the normal control rats (1.40 ± 0.10 vs 1.75 ± 0.05 mg/dl) ($p<0.05$). In diabetic rats orally administered 150 and 350 mg/kg of the mixed extract, significant reduction in the serum triglyceride concentration occurred on day 7, the reduction continued on other days up to day 28, but such changes were not significant ($p>0.05$) compared to day 0.

In the 350 mg/kg mixed extract concentration, significant reductions in the serum triglyceride concentrations occurred on days 7 and 14; the decline continued on days 21 and 28 but were not significant compared to the day 0 value ($p>0.05$). Similar trend were recorded in diabetic rats treated with glibenclamide.

Effects of the aqueous extracts of the combined plant extracts on the high density lipoprotein concentration (mg/dl)

Changes in the high density lipoprotein (HDL) of rats treated with varying doses of the mixed extracts were not significant compared to the normal, drug-treated, and the untreated controls on day 0 ($p>0.05$). By day 7 (Table 7), rats administered 250 mg/kg of the mixed extracts had significantly lower HDL concentration compared to the normal, and drug-treated controls ($p<0.05$). Duration-dependent changes in the serum HDL concentrations were not recorded in other days of the experiment in all the experimental groups when compared to the respective day 0 concentrations ($p>0.05$).

Effects of the aqueous extracts of the combined plant extracts on the low density lipoprotein concentration (mg/dl)

Changes in the low density lipoprotein (LDL) concentration of the rats treated with varying dosages of the mixed extracts on day 0 were significantly lower when compared to the diabetic untreated rats ($p<0.05$) (Table 8). Compared to other controls, the variations were similar ($p>0.05$). For the remaining days of the experiment, the LDL concentrations of the mixed extract-treated rats did not vary significantly when compared to the normal and drug-treated controls ($p>0.05$). Duration-wise, all the experimental groups (treatment and controls alike) had no significant variations in the low density lipoprotein concentrations on sampling days compared with respective day 0 values ($p>0.05$), despite slight variations in the mean low density lipoprotein cholesterol concentration recorded.

Discussion

Phytochemical constituents demonstrated in the leaves were reducing sugar and phenol (which are significant in *O. gratissimum*), tannin, soluble carbohydrate, hydrogen cyanide, steroids; alkaloids, flavonoids, saponins, glycosides and terpenoids (which are significant in *G. latifolia*). Flavonoids and polyphenols components of plants are well known antioxidants [26]. Besides these antioxidant properties, the phenols are reported to inhibit alpha-

amylase and sucrose, as well as the action of sodium glucose-transporter 1 (S-GLUT-1) of the intestinal brush border, hence their antidiabetic action. Isoflavones, tannins, chlorigenic acids and crude saponins possess potent S-GLUT-1 mediated inhibition of glucose transport, hence antidiabetic activity [26]. Yoshikawa *et al*. [8] also reported in their work that polyphenols suppress post-prandial hyperglycaemia and glucose transport across the small intestine. More so, saponin delays glucose transfer from the stomach to the small intestine [9, 10]. Bnouham *et al*. [12] reported that plant flavonoids exert their antidiabetic activity via antioxidant properties.

In the present study, significant decreases were recorded in the body weight of rats in the treatment (i.e. extract and drug) groups during the experiment. This result disagreed with the work of Akpaso *et al*. [27] and Effiong *et al*. [28]. The decrease in weight may be due to the loss in muscle and adipose tissue resulting from excessive breakdown of tissue protein and fatty acids [29]. Glycosuria is known to cause a significant loss of calories for every gram of glucose excreted and presumably this loss results in severe weight loss in spite of increased appetite. The unpalatability of the *G. latifolia* due to the presence of bitter substances in the leaves, may have reduced the quantity of food consumed by the rats, hence their weight decrease [28].

Telfaria occidentalis increased the weight of animals that consumed it for a long term due to its palatability [30]. However, weight gain was recorded in rat groups treated with the mixed extracts towards the end of the experiment. This study was in agreement with the earlier study of Nimenibo-Uadia and Osagie [31] that reported that rats treated with the mixed extract tended to gain more weight than the drug (gilbenclamide) treated group. Severe weight loss was prevented in the extract treated group probably due to interaction of several bioactive compounds. Overweight not only increases mortality, but is also associated with many chronic diseases such as high blood pressure, cardiovascular diseases, hypercholesterolemia, diabetes and increased likelihood of cancer [32]. In this study, combined leave extracts of *O. gratissimum* and *G. latifolium* significantly reduced the blood glucose levels of the experimental rats. This agreed with the work of Okokon *et al.* [33] which reported that the combined extracts of *G. latifolium* and *O. gratissimum* reduced the blood glucose level of the diabetic rats. The result also agreed with the works of Aguiyi *et al*. [34] and Egesie *et al*. [35]. Both reported the efficacy of *O. gratissimum* in lowering the blood glucose level in STZ-induced diabetic animals. Akah *et al*. [36] reported that intraperitioneal injection of both methanolic and aqueous extracts of *G. lafifolium* exhibited a significant antidiabetic effect by ameliorating alloxan-induced increase in blood sugar of rats. The result of this study also agreed with the works of Akpaso *et al*. [27] and Obi *et al.* [37]. Plant extracts contain phytochemicals including tannins, saponins, polyphenols and alkaloids which contribute to their blood sugar lowering effect [26]. Extract treated groups at dose 250 and 350 mg/kg showed lower blood glucose level than those treated at dose 100 mg/kg and drug treated. In general, the extract treated groups had lower blood sugar level than the drug treated. This proves the efficacy of polyherbal therapy. The serum cholesterol concentration of rats in the treated groups did not differ significantly from those of the control. This could be as a result of the diet. The rats used for this study were fed chicken grower's mash which was not meant to fatten the animals. Increase in total cholesterol concentration promotes the incidence of coronary heart disease [38]. No significant changes were recorded on the triglyceride concentration of extract treated rats compared to normal control, despite some slight fluctuations. The high density lipoprotein concentration of rats fed mixed extracts did not differ significantly from the control, although, there was a slight increase. High density lipoprotein are carrier proteins that are believed to transport cholesterol away from blood vessels to the liver, and thus to offer some protection from atherosclerosis [32]. This showed that mixed extracts of *G. latifolium* and *O. gratissimum* may have protective function against the development of atherosclerosis.

Changes in the low density lipoprotein concentration of rats treated with varying doses of the mixed extracts did not differ significantly compared to the control. Low density lipoproteins are plasma proteins that transport triglycerides and cholesterol to the arteries [32]. They are believed to contribute to arteriosclerosis.

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Table 1. Qualitative and Quantitative phytochemical compositions of the aqueous leaves extracts of *Ocimum gratissimum* and *Gongronema latifolia*

Parameters	Plant Extracts			
	Qualitative		Quantitative	
	O. gratissimum	*G. latifolia*	*O. gratissimum*	*G. latifolia*
Tannin (mg/100g)	++	+++	6.87±0.005	10.15±0.002*
Reducing sugar (mg/100g)	+++	++	348.26±0.003*	230.44±0.002
Soluble carbohydrate (mg/g)	+	++	3.82±0.002	3.94±0.004*
Hydrogen cyanide (mg/g)	+	+	2.95±0.002	3.15±0.002*
Steroid (mg/g)	++	++	2.03±0.003	3.98±0.668
Alkaloid (mg/100g)	++	+++	5.39±0.002	6.90±0.002*
Terpenoid (mg/100g)	+	+	0.25±0.004	0.38±0.003*
Flavonoid (mg/100g)	++	++	4.43±0.003	4.52±0.035
Saponin (mg/g)	+	+	0.86±0.002	0.87±0.002
Phenol (mg/100g)	++	++	11.90±0.001*	10.74±0.003
Glycoside (mg/g)	+	+	0.56±0.003	0.66±0.002*

+ = slightly present; ++ = highly present; +++ = present in greatest amount, Mean values with asterix as superscript are significant ($p < 0.05$)

Table 2. Number of deaths recorded during LD_{50} determination

Dose of extract (mg/kg)	Number of animals used	Mortality
10	3	0
100	3	0
1000	3	0
1600	3	0
2900	3	0
5000	3	0

Table 3. Effects of the aqueous extracts of the combined plant extracts on the body weight (kg) of rats

Extracts concentration (mg/kg)	Duration (Days)				
	0	**7**	**14**	**21**	**28**
Normal Control	196.38±3.75[1]	200.62±2.73[1]	202.21±3.77[1]	205.30±3.75[1]	202.04±6.05[1]
150 (mg/kg)	196.99±10.22[1]	176.32±11.00[1]	179.10±10.66[1]	181.4133±8.48[1]	185.67±8.30[1]
250 (mg/kg)	199.79±6.12[1]	183.95±8.46[1]	183.96±8.35[1]	186.94±7.70[1]	189.74±4.74[1]
350 (mg/kg)	205.01±15.71[1]	187.18±12.74[1]	190.12±13.46[1]	189.70±13.59[1]	195.75±15.72[1]
Drug Control	185.36±3.28[1]	178.68±3.42[1]	179.58±2.51[1]	179.92±2.82[1]	182.61±3.11[1]
Diabetic Control	202.49±4.01[1]				

Mean values with different numbers as superscript in a row are significantly different ($P < 0.05$)

Table 4. Effects of the aqueous extracts of the combined plant extracts on the blood glucose concentration (mg/dl)

Extract Concentration (mg/kg)	Duration (days)				
	0	**7**	**14**	**21**	**28**
Normal Control	81.35±0.03^{d1}	77.34±0.68^{c12}	75.89±0.23^{c12}	78.34±0.23^{c1}	79.65±0.76^{c1}
150 (mg/kg)	275.93±6.36^{b1}	101.00±0.00^{a2}	98.56±0.87^{a2}	96.67±0.23^{ab2}	99.45±0.67^{a2}
250 (mg/kg)	256.53±3.21^{c1}	86.67±0.21^{bc2}	86.67±0.67^{bc2}	89.12±0.45^{bc2}	90.32±1.67^{bc2}
350 (mg/kg)	243.60±2.45^{cd1}	88.33±0.34^{bc2}	88.33±0.45^{bc2}	88.12±0.56^{bc2}	89.33±1.67^{bc2}
Drug Control	328.47±2.67^{a1}	101.00±0.67^{a2}	101.00±0.11^{a2}	98.59±0.45^{a3}	100.21±0.67^{a2}
Diabetic Control	213.20±0.07^{d1}	0	0	0	0

Mean value with different alphabets as superscript in a column are significantly different (P < 0.05). Mean value with different numbers as superscript in a row are significantly different (P < 0.05).

Table 5. Effects of the aqueous extracts of the combined plant extracts on the total cholesterol concentration (mg/dl)

Experimental groups	Duration (Days)				
	0	**7**	**14**	**21**	**28**
Normal Control	3.00±0.00^{a12}	3.15±0.05 ^{a12}	2.80±0.60 ^{a2}	4.10±0.00 ^{a1}	3.20±0.20 ^{a12}
150 (mg/kg)	3.32±0.06 ^{a1}	3.10±0.00 ^{a1}	2.70±0.20 ^{a1}	3.70±0.10 ^{a1}	2.90±0.10 ^{a}
250 (mg/kg)	3.06±0.01 ^{a1}	2.80±0.70 ^{a1}	2.75±0.15 ^{a1}	3.50±0.40 ^{a1}	2.85±0.25 ^{a1}
350 (mg/kg)	3.07±0.02 ^{a12}	3.05±0.15 ^{a12}	2.75±0.15 ^{a2}	3.65±0.05 ^{a1}	3.05±0.35 ^{a12}
Drug Control	2.98±0.07 ^{a2}	3.25±0.05 ^{a2}	2.95±0.05 ^{a2}	4.20±0.00 ^{a1}	3.25±0.15 ^{a2}
Diabetic Control	3.20±0.02 ^{a}	0	0	0	0

Mean value with different alphabets as superscript in a column are significantly different (P < 0.05). Mean value with different numbers as superscript in a row are significantly different (P < 0.05).

Table 6. Effects of the aqueous extracts of the combined plant extracts on the triglyceride concentration (mg/dl)

Experimental groups	Duration (days)				
	0	**7**	**14**	**21**	**28**
Normal Control	1.60±0.00^{ab2}	0.85±0.05 ^{a3}	1.00±0.00 ^{b3}	1.75±0.05 ^{a1}	1.45±0.05 ^{a2}
150 (mg/kg)	1.75±0.07 ^{ab1}	0.90±0.10 ^{a2}	1.25±0.15 ^{b12}	1.60±0.10 ^{ab12}	1.80±0.40 ^{a1}
250 (mg/kg)	1.55±0.07 ^{bc1}	0.95±0.05 ^{a2}	1.65±0.15 ^{a1}	1.40±0.10 ^{b1}	1.65±0.05 ^{a1}
350 (mg/kg)	1.65±0.07 ^{ab1}	0.95±0.05 ^{a3}	1.35±0.05 ^{ab2}	1.55±0.05 ^{ab12}	1.60±0.10 ^{a1}
Drug Control	1.70±0.14 ^{ab1}	0.85±0.05 ^{a2}	1.30±0.00 ^{ab2}	1.45±0.05 ^{b1}	1.80±0.30 ^{a1}
Diabetic Control	1.40±0.00 ^{c}	0	0	0	0

Mean value with different alphabets as superscript in a column are significantly different (P < 0.05). Mean value with different numbers as superscript in a row are significantly different (P < 0.05).

Table 7. Effects of the aqueous extracts of the combined plant extracts on the high density lipoprotein concentration (mg/dl)

Experimental groups	Duration (days)				
	0	7	14	21	28
Normal Control	0.35±0.05^{a1}	0.70±0.14 ^{a1}	0.60±0.20 ^{a1}	0.50±0.10 ^{a1}	0.45±0.05 ^{a1}
150 (mg/kg)	0.40±0.00 ^{a1}	0.50±0.00 ^{ab1}	0.55±0.05 ^{a1}	0.55±0.25 ^{a1}	0.35±0.05 ^{a1}
250 (mg/kg)	0.35±0.05 ^{a12}	0.30±0.00 ^{b2}	0.60±0.10 ^{a1}	0.50±0.10 ^{a12}	0.45±0.05 ^{a12}
350 (mg/kg)	0.45±0.05 ^{a1}	0.55±0.07 ^{ab1}	0.85±0.05 ^{a1}	0.55±0.25 ^{a1}	0.80±0.00 ^{a1}
Drug Control	0.35±0.05 ^{a}	0.70±0.14 ^{a1}	0.90±0.00 ^{a1}	0.60±0.00 ^{a1}	0.70±0.40 ^{a1}
Diabetic Control	0.30±0.00 ^{a}	0	0	0	0

Mean value with different alphabets as superscript in a column are significantly different (P < 0.05). Mean value with different numbers as superscript in a row are significantly different (P < 0.05).

Table 8. Effects of the aqueous extracts of the combined plant extracts on the low density lipoprotein concentration (mg/dL)

Experimental groups	Duration (days)				
	0	7	14	21	28
Normal Control	1.80±0.00 ^{b1}	1.90±0.00 ^{a1}	1.65±0.35 ^{a1}	2.10±0.10 ^{a1}	1.80±0.10^{a1}
150 (mg/kg)	1.75±0.05 ^{b1}	2.05±0.05 ^{a1}	1.50±0.10 ^{a1}	1.55±0.15 ^{a1}	1.65±0.35^{a1}
250 (mg/kg)	1.85±0.05 ^{b1}	1.67±0.35 ^{a1}	1.30±0.10 ^{a1}	1.30±0.30 ^{a1}	1.55±0.25^{a1}
350 (mg/kg)	1.75±0.05 ^{b12}	1.90±0.20 ^{a1}	1.20±0.10 ^{a2}	1.30±0.10 ^{a12}	1.45±0.25^{a12}
Drug Control	1.80±0.10 ^{b1}	1.95±0.15 ^{a1}	1.35±0.05 ^{a1}	2.25±0.45 ^{a1}	1.65±0.25^{a1}
Diabetic Control	2.15±0.05 ^{a}	0	0	0	0

Mean value with different alphabets as superscript in a column are significantly different (P < 0.05). Mean value with different numbers as superscript in a row are significantly different (P < 0.05).