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Effect of *Gongronema latifilium* and *Luffa ægyptiaca* decoction on the blood glucose, lipids and liver enzymes of alloxan induced diabetic rats

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Abstract

There is lack of information on the use of *Gongronema latifilium* and *Luffa aegyptiaca* leafy vegetables decoction in the management of diabetes. The study investigated the effect of *G. latifilium* and *L. aegyptiaca* leafy vegetable decoction on blood glucose level, lipid profile and liver enzymes of alloxan-induced diabetic wistar rats. Experimental design was adopted to randomly assign 30 rats of 5 treatment groups and 1 untreated group each. The leafy vegetables were purchased, picked separately and washed with deionized water. The vegetables were separately boiled for 10.3minutes in 3.5L and 4.5L of water, respectively. The decoctions were sieved out separately and stored in the refrigerator for further study. Alloxan was used to induce diabetes in the rats, while treatment lasted for 21days. Phytochemicals, acute toxicity test, blood glucose and liver function test were done using standard methods. Pair-sample t-test ($p < 0.05$) was used to determine the effect of the decoctions and percentage difference was calculated. There were 69.64% and 68.88% decreases in the blood glucose level of diabetic rats treated with 3mL/kg bodyweight of *G. latifilium* and *L. aegyptiaca* decoctions, respectively. There was significant increase ($p < 0.05$) in the HDL-C level of diabetic rats after treatment with 3mL/kg bodyweight of *G. latifilium* and *L. aegyptiaca* decoctions. Significant decrease ($p < 0.05$) was observed in the liver enzymes activity of the diabetic rats after treatment with 3mL/kg bodyweight of *G. latifilium* and *L. aegyptiaca* decoctions. The leafy vegetables decoction had potentials to reduce high blood glucose level, dyslipidemia and, for normal liver function.

Keywords: *Gongronema latifilium*; *Luffa aegyptiaca*; decoction; blood glucose; diabetic rats; liver enzymes; dyslipidemia

Introduction

Diabetes mellitus is a chronic disorder of energy (carbohydrate, protein and fat) metabolism, which is becoming increasingly common, resulting from deficient action and secretion of insulin on target skeletal muscle, tissue and/or liver (1). The World Health Organization (WHO) (2) reported that global population is in the midst of diabetes epidemic. The sub-Sahara African populace are under greater risk probably due to adoption of westernize diet and lifestyle. This is facilitated by advertisement for consumption of unhealthy foods and inactivity (3). Chronic illnesses like diabetes mellitus, hypertension, cancer, and renal failures are major problems of many people in the world. Systemic hypertension and diabetes mellitus are common chronic diseases that frequently co-exist and can significantly affect the health care need and clinical outcome of the affected individual. Most of the chronic diseases are the principal causes of death in the developed and developing countries. Diabetes mellitus is a complex syndrome that causes a lot of physiological changes including alteration in carbohydrate (CHO), protein and fat metabolism. Diabetes, being a chronic disease, unlike the communicable diseases, is often a slow, insidious process. Therefore, uncontrolled diabetes may result in long term damage, dysfunction and failure of various organs especially the heart, kidney and eyes.

Despite numerous advances in the field of Medical and Human Nutrition complication associated with poor glycaemic control is on the increase. Each year the orthodox drugs become increasingly ineffective in 5% to 10% of treated patients and, over ten years, they remain effective in only 20% of those patients initially responsive. Furthermore, existing orthodox hypoglycemic therapies are generally ineffective in about 20% of Type 2 cases, due to non-compliance with diet and impaired pancreatic function. Also the few western (orthodox) treatments available are not just often very expensive, associated with severe side effects but also require rigorous training to administer them effectively. All these interventions are not readily affordable. Following the inefficiency of modern medicine to combat the scourge of diabetes, alternative strategies are urgently needed (4). It is not uncommon in the developing countries for patients to use herbs for conditions such as hypertension, diabetes and weight loss despite unproved effect on lowering blood sugar. Many patients use plant remedies as first line treatment for many chronic ailments including diabetes. This paradigm shift is also promoted by the fact that WHO has authenticated the use of food-based remedies for the treatment of diabetes, and that estimated 80% of the world's population use botanical medicine for their primary health care needs (4).

In spite of strong arguments supporting the use of plants with medicinal properties, a lot of mist is associated with the use of traditional remedies. It is argued that some plants in use may not be efficient. Therefore, using traditional remedies that have no proven clinical benefits to patients may lead to delays in seeking appropriate treatment leading to severe diabetes related complications and associated disability and mortality (5). There is urgent call for in-depth studies to discover the actual medicinal potentials of the indigenous foods commonly used in Nigeria for treatment of diabetes. Lack of information on the indigenous plants as consumed puts a big question mark on their use as remedy for the treatment of diabetes. In Nigeria several edible plants have been identified within the forest and savanna region for the treatment of diabetes. Among them are a wide variety of trees and herbs that are of medicinal value ranging from the popular and highly utilized ones to the less popular and under-utilized ones. The underutilized ones include bush buck (*Gongronema latifilium*), a tropical rainforest plant mainly used as vegetable, medicine or spice by the people, Egyptian cucumber (*Luffa aegyptiaca*): which are excellent fruit in nature containing many essential constituents required for good health. Studies have been done on extracts of these leafy vegetables in the management of diabetes, however, information is lacking on their use in form of decoction in the management of diabetes. The study was designed to investigate the effects of *Gongronema latifilium* and *Luffa aegyptiaca* leafy vegetable decoction on blood glucose, lipids and liver enzymes of alloxan induced diabetic male Wister rats. This will provide evidence-based information on the potential use of the leaves decoction as a cost effective therapy.

Methodology

Experimental design was adopted for the study. *Gongronema latifilium*, and *Luffa aegyptiaca* leafy vegetables were used for the study. The leafy vegetables were collected from their natural habitats at Umuabi-Udi Local Government Area of Enugu State, Nigeria. Matured leaves were used, and the leaves were authenticated by a Taxonomist in the Department of Plant Science and Biotechnology, University

of Nigeria, Nsukka, Enugu State, Nigeria. Samples were prepared by the researcher within one hour of collection.

Sample preparation

The researcher paid preliminary visits to the President of defunct Umuabi General Assemble and Umuabi Progressive Union, respectively. Through them the researcher contacted the village heads and leaders. With the help of the village heads and leaders the researcher contacted the users of plant remedies in management of diabetes. Many of the users were un-cooperative, very secretive and repelled participating in the research. However, with the help of the village heads and leaders the researcher finally got three users per plant. The six users agreed to participate in the research on condition that they will be visited individually to demonstrate the preparation methods. The condition was accepted by the users of the plants.

The researcher then visited the three users per plant and watched them individually preparing the green leafy vegetables decoction. After the initial visits the researcher made arrangement with the individual users on when to come with some necessary equipment for better reportage. The equipment included measuring jug, weighing scale and stop watch. On each user's appointment day the researcher visited the user with measuring jug, weighing scale and stop watch in company of research assistants.

On each visiting day, the researcher with the help of the research assistants measured the quantity of each green leafy vegetable used using a weighing scale, the volume of water used for boiling it with measuring jug and the time taken to boil it using a stop watch. At the end of the whole visits it was observed that different quantities or measures of *Gongronema latifilium* and *Luffa aegyptiaca* leafy vegetables respectively, were used by the three different users. Generally, they were separately picked to remove extraneous materials and washed with deionized water. Also, they were separately prepared by boiling in water for some minutes and the decoction sieved out for consumption. The measures of green leafy vegetables used, the quantity of water used, as well as the boiling time differed slightly from the three users. The samples were then prepared using the method of preparation used by the three users, as shown in below Table.

Name of leaf	***Variable***	***User 1***	***User 2***	***User 3***	***Average use***
Gl	QLU	3.2 kg	3 kg	2.8 kg	3 kg
	VWU	3litres	4litres	3.5 litres	3.5litres
	BT	12mins	9mins	10mins	10.3mins
	QC	280	300	315	298.3ml
La	QL U	4.7kg	4,3kg	4kg	4.3kg
	VWU	4,5litres	4.3litres	4 litres	4.3litres
	BT	10mins	11mins	10mins	10.3mins
	QC	320ml	300ml	295ml	305ml

Gl = *Gongronema latifolium*; La = *Luffa aegyptiaca*; QLU = Quantity of leafy vegetables used; VWU =Volume of water used for boiling; BT = Boiling time; QC= Quantity consumed.

Table: Preparation of *Gongronema latifilium* and *Laffa aegyptiaca* leafy vegetable decoction by the three users.

Preparation of samples for rat study

The quantity of the leafy vegetable, volume of water, boiling time and quantity consumed as displayed on Table 1 above were used for the rat study. The vegetables were separately picked to remove extraneous materials and washed with deionized water. The leafy vegetables were separately boiled with local tripod (*ekwu*) using aluminum pot, in 3.5L and 4.3L of water, for *Gongronema latifilium*, and *Luffa aegyptiaca*, respectively, and boiled for 10.3 minutes. The end of boiling point coincided with the leaf colour change from green to brown. The decoctions were sieved out separately using muslin cloth and then, stored in the refrigerator for analysis and rat study.

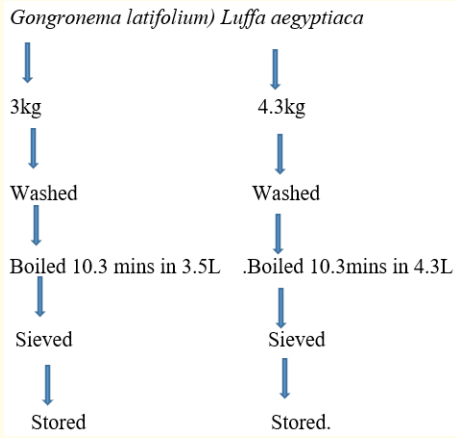

Figure 1: A flow chart for preparation of samples for analysis and rat study.

NB. For each sample 1 litre was used for analysis while the rest was used for rat study.

Chemical analysis

Alkaloid determination

Alkaloid determination was done using method described by Harborne (6). Five (5 ml) of each sample was weighed and added into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4hours. Each sample was filtered and the extract concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium was added drop wise to each sample and precipitate. Each whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

Flavonoid determination

This was done using the method described by Bohm and Kocipal-Abyazan (7):

Ten (10 ml) of each sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. For each sample, the whole solution was filtered through whatman filter paper No 42 (125mm). Each filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Determination of total phenols

Total phenol was done using the method of Olivera, (8).

Fat free samples of each were boiled with 50ml of ether for the extraction of the phenolic component for 15min. Five (5ml) of each was pipette into a 50ml flask, and then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5ml of concentrated amylalcohol were also added to each. Each sample was made up to mark and left to react for 30min for colour development. Each was measured at 505nm. Milligram

Determination of carotenoids

The method according to Harbone (6) was used. Five Milliliters (5ml) of each sample was homogenized in 1% methanol solution using lab blende. Each homogenate was filtered to obtain the initial crude extract. 20ml of ether was added to each filtrate to take up

the carotenoid. 20ml of distilled water was also added and the mixtures shaken very well to separate carotenoid. A 20ml of distilled water was evaporated to dryness at low temperature 30-50 degrees Celsius. Each dry extract was then saponified with ethanoic potassium hydroxide and left overnight in a dark cupboard. The next day, the carotenoids extract (either layer) of each sample was dried in desiccators. Each was then treated with a light petroleum sprit and allowed to stand overnight in a freezer (-10°C). The next day, the precipitated steroid from each sample was removed by centrifugation and the carotenoids extract was evaporated to dryness in a weighed evaporating dish. Each dish was cooled in desiccator, weighed and carotenoid expressed as a parentage of the sample weight.

Acute toxicity test (lethal Dose, LD_{50}) of the plants

Acute toxicity or lethality of *Gongronema latifilium*, and *Luffa aegyptiaca*, green leafy vegetables decoction was carried using Lorke (9) with some modification. Acute toxicity of the leafy vegetables decoction was carried out using 36 mice. The mice were divided into two groups. Each group was further divided into 6 groups of 3 mice each.

In the first phase 1ml, 2 ml and 3 ml respectively, of *Gongronema latifilium*, and *Luffa aegyptiaca* green leafy vegetables decoction kg/ body weight, respectively, was given to the first 6 groups (18 mice) in this order; A_1, A_2 and A_3 received 1ml, 2 ml and 3 ml of *Gongronema latifilium* / kg body weight respectively and B_1, B_2 and B_3 received 1ml, 2 ml and 3 ml of *Luffa aegyptiaca* / kg body weight respectively. They were observed for 24hours no mortality or behavoural change was observed in any of the groups within the 24 hours.

In the second phase 4ml, 5.ml and 6ml respectively, of *Gongronema latifilium* and *Luffa aegyptiaca* green leafy vegetables decoction kg/body weight respectively, was given to the second 6 groups (remaining 18 mice) in this order; A_4, A_5 and A_6 received 4ml, 5 ml and 6 ml of *Gongronema latifilium* / kg body weight respectively, and B_4, B_5 and B_6 received 4 ml, 5 ml and 6 ml of *Luffa aegyptiaca* / kg body weight respectively. The mice were observed closely for 24 hours for behavioural changes or death. Table 1 and Table 2 shows the outcome of the toxicity test.

Grp	*No of rats*	*Vegetable decoction*	*Amount /kg b.w*	*Reaction*
A_1	3	*Gongronema latifolium*	1ml	No reaction
A_2	3	*Gongronema latifolium*	2ml	No reaction
A_3	3	*Gongronema latifolium*	3ml	No reaction
B_1	3	*Luffa aegyptiaca*	1ml	No reaction
B_2	3	*Luffa aegyptiaca*	2ml	No reaction
B_3	3	*Luffa aegyptiaca*	3ml	No reaction

Table 1: First phase of acute toxicity test of *Gongronema latifilium* and *Luffa aegyptiaca*, green leafy vegetables decoction using 36 mice.

Grp	*No of rats*	*Vegetable decoction*	*Amount /kg b.w*	*Reaction*
A_4	3	*Gongronema latifolium*	4ml	No reaction
A_5	3	*Gongronema latifolium*	5ml	1 weak
A_6	3	*Gongronema latifolium*	6ml	1 big toe oedoma
B_4	3	*Luffa aegyptiaca*	4ml	No reaction
B_5	3	*Luffa aegyptiaca*	5ml	2 sluggish
B_6	3	*Luffa aegyptiaca*	6ml	1 died

Table 2: Second phase of acute toxicity test of *Gongronema latifilium* and *Luffa aegyptiaca*, green leafy vegetables decoction using 36 mice.

Animal study

Ethical consideration

All animals for the experiment were handled according to the international guiding principles for research involving animals as permitted by the University of Nigeria Ethical Committee concerning the use of laboratory animals.

Animal sourcing and housing

The experimental animals for the study were thirty (30) healthy adult male Wistar rats weighing 230g - 250g, aged 3months. They were purchased from the Faculty of Veterinary Medicine, University of Nigeria Nsukka, and were kept in metallic laboratory cage in the Department of Nutrition and Dietetics, University of Nigeria, Nsukka. The animals were maintained under standard (room) temperature (28°C), humidity and 12 h light/dark cycle. They were allowed to acclimatize for 5days and fed with vital feeds (grown maxx) and distilled water *ad libitum* during the study period. they were housed in groups of five (5) in metallic cages specially designed to separate feaces and urine. The rat dose for the treatments (leafy vegetables decoction and oral hypoglycemic drug) were calculated based on the body weight using the LD_{50} results as a guide, thus;

$$\frac{\text{dosage}}{\text{1kg (1000g)}} \times \frac{\text{weight of rat}}{1}$$

Experimental design

The study period was twenty-nine (29) days, 5 days for acclimatization, 1 day for inducing diabetes, 2 days for establishment of diabetes and 21 days for giving the experimental treatment. Data including weight were collected from all the groups on day 6. When diabetes was established on day 8 the baseline data were collected and treatment commenced with the green leafy vegetable decoctions. Groups A and B were given 2 ml of *Gongronema latifilium* and *Luffa aegyptiaca* leafy vegetables decoction per kg bodyweight, respectively. Also, groups C and D were treated with 3 ml of of *Gongronema latifilium* and *Luffa aegyptiaca* leafy vegetables decoction per kg bodyweight, respectively. Rats in group E (positive control) were induced and then, treated with pharmacological standard dose of Glibenclamide 0.5 mg/kg bodyweight, while rats in group F (negative control) were induced but not treated at all to see if they will recover spontaneously. The treatments (green leafy vegetables decoction and oral hypoglycaemic drug) were given orally every day using a syringe. During the study period blood samples were collected on days 6, 8 and 29 to determine some biochemical parameters (blood glucose level, lipids and liver enzyme activities). After collecting blood samples on day 29, the rats were sedated with chloroform and sacrificed by decapitating them and the pancreas and liver harvested for histo-pathological examination. The result of the biochemical indices on day 8 served as the baseline data.

Diabetes induction

After the acclimatization period of 5 days, the rats were fasted overnight, and then induced diabetes using alloxan on day 6. Freshly prepared aqueous solution of alloxan monohydrate was injected intra peritoneally (IP) to the rats at a single dose of 150mg/kg body weight. The rats were allowed free access to 5% glucose solution to avoid possible effect of hypoglycemia. After 48 hours (2 days) of inducing diabetes, blood was taken from each rat to confirm diabetes. The determination was done using standard method. The result was reported as mg/dl. Rat with blood glucose concentration of l30mg/dl and above was considered diabetic.

Feeding trial

Wistar rats (n = 30) were divided into six (6) groups of 5 rats each group labeled A - F. The rats in groups A and B were given 2 ml of *Gongronema latifilium* and *Luffa aegyptiaca* leafy vegetables decoction per kg bodyweight, respectively. Groups C and D received 3 ml of *Gongronema latifilium* and *Luffa aegyptiaca* leafy vegetables decoction per kg bodyweight. Rats in group E (positive control) were treated with Glibenclamide 0.5 mg/kg body weight. Rats in group F (negative control) were induced but not treated at all. The

treatments (green leafy vegetables decoction and oral hypoglycaemic drug) were given orally every day using syringe while distilled water and rat chow vital feeds (grown maxx) were given *ad libitium* to all groups.

Groups	***No of rats***	***Experimental diet***	***Quantity (/kg BW)***
A	5	Rat chow+ water + *Gongronema latifilium*	2 ml
B	5	Rat chow + water+ *Luffa aegyptiaca*	2 ml
C	5	Rat chow + water + *Gongronema latifilium*	3 ml
D	5	Rat chow + water + *Luffa aegyptiaca*	3 ml
E	5	Rat chow + water + Glibenclamide	0.5 mg
F	5	Rat chow + water	

Table 3: Experimental diet.

Blood sample collection

Nucleo capillary tube was carefully inserted into the lateral canthus to puncture the retro bulbar plexus to enable outflow of about 2ml of blood into a clean glass test tube. The blood samples were kept at room temperature (27 – 28°C) for 30 minutes to clot. Afterwards the test tubes containing the clotted blood samples were centrifuged at 300 revolutions per minute for ten minutes using a table centrifuge to enable a complete separation of the serum from the clotted blood. The clear serum supernatant was carefully aspirated with syringe and needle into a clean sample bottle for the biochemical analysis. Blood samples were collected on days 6, 8 and 29.

Determination of blood glucose

Determination of blood glucose was done by the Trinders glucose-oxidase principle by Lott & Tuner, (10) using the one Touch Basic (Life-scan Milpitas, CA) instrument.

Method: The test strip was inserted in the meter. The meter turns on automatically. A small drop of blood was put on the top white edge of the test strip. The test strip automatically draws the blood into the reaction cell where the reaction takes place. The blood glucose level was read on the meter.

Lipids determination

Determination of Serum High Density Lipoprotein

Dextran sulphate-mg (II) method for the in-vitro determination of HDL-cholesterol in serum, using Quimica Clinica Applicada (QCA) HDL test kit was used to determine HDL (11).

Method

Accurately 0.03m1 of the serum sample was added to a clean labeled 1ml test tube. A drop of the precipitant solution was added to 1ml test tube. It was mixed and allowed to stand for 15 minutes at room temperature, centrifuged at 300 revolutions per minutes for 10 minutes.

Approximately 1ml of cholesterol working reagent was added to a set of cleaned test tubes. Two of the test tubes were labeled Standard 1 and 2 while the other was label blank apart from the test samples that were labeled according to group names and numbers (samples A, B, C to O). Approximately 0.01ml of the supernant derived from centrifugation of the precipitant-serum sample mixture was added to the test tube. It was mixed well and allowed to stand for 10 minutes at room temperature. Also 0.01ml of the standard was also added to each of the test tubes labeled standard I and 2. It was mixed well and allowed to stand for 10 minutes at room temperature. Reading was taken at absorbance of the samples and standard against the reagent blank at 52 5nm.

Cholesterol content of each sample was calculated using the following formular:

Calculations

The HDL cholesterol concentration in the sample were calculated using the following general formula:

$$\frac{A_{sample}}{A_{standard}} \times 52.5 = \text{mg/dl HDL - Cholesterol}$$

The result was expressed in mg HDL-cholesterol/dl.

Low density lipoprotein-cholesterol concentration was determined using QCA commercial kit, method of (12)

Principle

Low density lipoprotein-cholesterol (LDL-cholesterol) was determined from the difference between total cholesterol and cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethyleneglycol monomethyl ether.

LDL-cholesterol = Total cholesterol - cholesterol in the supernatant.

Reagents

Content	***Initial Concentration of Solutions***
1. Precipitation Reagent:-	
Polyvinyl sulphate	0.7 g/L
EDTA Na_{2s}	5.0 Mm
Polyethyleneglycol monomethyl ether	170 g/L
Stabilizers	

Procedure

Precipitation reaction: The precipitation solution (3 drops or 0.1 ml) was carefully measured into test tubes labeled accordingly. The serum sample (0.2 ml) was added to the labeled test tubes. The contents were thoroughly mixed and left to stand for 15 minutes at room temperature (20-25°C). Then, the mixture was centrifuged at 2,000 × g for 15 minutes and the cholesterol concentration in the supernatant was determined.

Cholesterol determination

The concentration of the serum total cholesterol was determined according to the QCA CHOD-PAP method (11).

Calculations

The LDL-cholesterol concentration in the sample was calculated using the following general formula:

LDL-cholesterol (mg/dl) = Total cholesterol (mg/dl) - 1.5 × supernatant cholesterol (mg/dl).

Determination of triacylglycerol concentration (Randox Enzyme Kit)

Principle (13)

The concentration of triacylglycerols were determined after enzymatic hydrolysis with lipases. The indicator showed quinoneimine formed from hydrogen peroxide, 4 - aminophenazone and 4 - chlorophenol under the catalytic influence of a peroxidase.

$$\text{Triacylglycerols} + H_2O \xrightarrow{\text{Lipases}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{glycerol} - 3 - \text{phosphate} + \text{ADP}$$

$$\text{Glycerol} - 3 - \text{phosphate} + O_2 \xrightarrow{\text{GPO}^*} \text{dihyroxy acetone} + \text{Phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + HCl + 4H_2O$$

GPO = Glycerol –3– phosphate oxidase

Reagents

Contents	***Initial Concentration of Solution***
Buffer	
Pipes buffer	40.0mmol/1, pH 7.6
4 – Chlorophenol	5.5mmol/1
Magnesium ions	17.5mmol/1
Enzyme Reagent	
4 – Aminophenazone	0.5mmol/1
ATP	1.0mmol/1
Lipases	≥1.5U/ml
Glycerol kinase	≥0.4U/ml
Glycerol – 3 – phosphate oxidase	≥ 1.5U/ml
Peroxidase	≥0.5U/ml
Standard	2.29mmol/1(200mg/dl)

Procedure

Three sets of tubes labelled reagent blank (B), standard (ST), and sample (S) were set up. The enzyme reagent (15ml) was reconstituted with 15ml of the buffer solution and the new solution stored in the refrigerator. An aliquot of the serum sample, 10.0µl was pipetted into the test tube labeled S while 10.0µ of the standard was pipetted into the test tube labeled ST. Then, 1.0ml of the reconstituted enzyme reagent was added to each of the three sets of test tubes. The contents of the test tubes were mixed and incubated in a water bath for 5 minutes at 37°C. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured at 500nm against the reagent blank within 60 minutes. The concentration of triacylglycerols in the serum samples were calculated using the formula:

$$\text{TAGs concentration} = \frac{A_{Sample}}{A_{S\,\text{tan}\,dard}} \times 2.29\text{mmol}/1$$

Determination of the total serum cholesterol

Determination of total cholesterol was done using Quimica Clinica Applicada (QCA) (14).

Principle

The total cholesterol determination using QCA enzyme kit based on the assay principle that total cholesterol was determined after enzymatic hydrolysis and oxidation The indicator coloured quinonic derivative were formed from hydrogen peroxidase and 4 amino-antipyrine in the presence of p - hydroxybenzoic acid and peroxidase.

$$\text{Cholesterol - esters} + H_2O \xrightarrow{\text{cholesterol\_\_esterase}} \text{Cholesterol + fatty acid}$$

$$\text{Cholesterol} + H_2O + O_2 \xrightarrow{\text{cholesterol}} \text{Cholesterol} + H_2O_2$$

$$2H_2O + \text{4- Aminoantipyrine + p – Hydroxybenzoicacid} \xrightarrow{\text{peroxidase}} \text{coloured quinnonoic derivative} + 4H_2O.$$

Procedure

Blank (BL), sample (SA) and standard (ST) were put in three labelled text tubes.0.01ml of the serum sample was pippeted into the sample (SA) test tube. Also 0.01ml of the standard (ST) was introduced into the standard test tube with a corresponding addition of 1ml of working reagent into each of the test tube. The solution in the different sets of test tubes were well mixed and allowed to stand for 5 minutes at 37°C or 10 minutes at room temperature. The absorbance was read at the wavelength of Hg 546nm.

Calculation

The total cholesterol concentration in the sample was calculated using the following general formular:

$$\frac{\text{SA OD x 200}}{\text{ST OD}} = \frac{\text{mg/dl of total cholesterol}}{1}$$

Where: SA = sample; ST = standard; OD = optical density; 200 = constant.

To convert to SI units: (mg/100dl) x 0.0259mm0L/L.

Determination of liver enzymes

Determination of the aspartate aminotransferase (AST) activity

The activity of aspartate aminotransferase was assayed by the method of Reitman and Frankel (15) as outlined in the Randox kit used.

Procedure

0.1 ml each serum sample was pipetted into a test tube and only 0.1 ml of distilled water was pipette into blank test tubes. 0.5 ml of Reagent one (R1) containing phosphate buffer, L - aspartate and beta - oxoglutarate was pipetted into both the blank and each serum sample test tube respectively. Each entire reaction medium was well mixed and incubated for 30 minutes in a water bath at 37°C. Immediately after incubation, 0.5 ml of Reagent two (R2) containing 2,4-dinitrophenylhydrazine was added to each blank and the serum sample test tubes and allowed to stand for exactly 20 minutes at 25°C. Finally, 5.0 ml of 0.4 N sodium hydroxide solutions was added to both each blank and serum sample test tubes respectively and mixed thoroughly. Then the absorbance read at a wavelength of 546 nm after 5 minutes.

$$\text{AST} = \frac{\text{Abs of sample} - \text{abs of} - \text{blank}}{\text{Slope}}$$

Determination of the alanine aminotransferase (ALT) activity

The activities of alanine aminotransferase wer assayed by the method of Reitman and Frankel (15) as outlined in Randox Kit.

Procedure

The serum sample (0.1 ml) was pipetted into the samples test tube and 0.1 ml of distilled water pipette into blank test tube, 500 µl of the ALT substrate buffer solution containing phosphate buffer, L-alanine and α-oxoglutarate (R_1) were added. The entire reaction media were well mixed and incubated for 30 minutes in a water bath at 37°C and pH 7.4. Immediately after incubation, 0.5 ml of Reagent two (R_2) containing 2, 4-dinitrophenylhydrazine was added to the blank and sample test tubes. These were thoroughly mixed and allowed to stand for exactly 20 minutes at 25°C. Finally, 0.5 ml of sodium hydroxide solution was added to both the blank and serum samples test tubes respectively and mixed thoroughly. The absorbance was read at wavelength of 546 nm after 5 minutes.

$$\text{ALT} = \frac{\text{Abs of sample} - \text{abs of blank}}{\text{Slope}}$$

Alkaline phosphatase (ALP)

Assay of serum alanine phosphate (ALP) activity was determined using randox labratory reagent kit, Uk, 29 4qy based on method developed by Ritman and frankel (15).

Procedure

For each sample, 0.5ml of alkaline phosphatase substrate was dispense into labeled test tubes and equilibrate to 37°C for three (3) minutes. At timed intervals, 0.05mL (50 u1) of each standard, control, and sample was added to its respective test tube. Then it was mix gently. Deionized water was used as sample for Reagent Blank. Incubation was done for exactly ten (10) minutes at 37°C.

Following the same sequence as in Step 2, 2.5 mL Alkaline Phosphatase Color Developer was added at timed intervals. Then it was mixed well. The wavelength of the spectrophotometer was set at 590 nm. Zero with Reagent Blank. (Wavelength range 580-630). Absorbance of samples was read and recorded.

Calculations

The activity of alkaline phosphatase in the sample was calculated using the following formula:

$$\frac{\text{A Sample x C}_{\text{Standard}}}{\text{A standard}} = \text{C}_{\text{sample}}$$

Euivalent enzyme activity of standard =50 U.

Statistical analysis

Data collected were coded, keyed into the computer and analysis was done with IBM-statistical package for service solution (IBM-SPSS) version 23. The Results of the study were analysed using descriptive statistics and paired sample t-test was used to analysed the effect of treatment. Differences were significance at $p < 0.05$.

Results

Figure 1 shows the phytochemical content of the green leafy vegetables (*G. latifilium* and L. aegyptiaca) decoction as consumed (per 100 ml). The result showed that alkaloid content of *G. latifilium* was 0.31 mg/100ml, while *L. aegyptiaca* had 0.99 mg/100ml content

of alkaloid. *Luffa aegyptiaca* contained 12.60 mg/100ml of phenol, while *G. latifilium* had 5.10 mg/100ml of phenol. *Luffa aegyptiaca* had the highest (936.67 mg/100ml) content of carotenoid, while *G. latifilium* had 686.67 mg/100ml of carotenoid. Similarly, *Luffa aegyptiaca* had highest (1.70 mg/100ml) content of flavonoid, while *G. latifilium* had 1.17 mg/100ml of flavonoid.

Table 4 shows the effect of *G. latifilium* and *L. aegyptiaca* leafy vegetables decoction as consumed on the blood glucose level of the different diabetic rat groups. The result showed that only untreated group (negative control) had sustained increased blood glucose, baseline (218.60 mg/dL), end-line (324.80 mg/dL) with 48.58% increase in the blood glucose level of the rats. Groups of diabetic rats treated with 2ml/kg bodyweight of *G. latifilium* and *L. aegyptiaca* respectively, showed slight percentage decreases in the blood glucose level (62.51% and 61.92%, respectively). There were 69.64% and 68.88%, respectively, decreases in the blood glucose level of diabetic rats treated with 3ml/kg bodyweight of *G. latifilium* and *L. aegyptiaca* decoctions. Also the group of diabetic rats treated with 0.5ml/kg bodyweight of glibenclamide showed 57.71% decrease in the blood glucose level.

Table 5 shows the effect of *Gongronema latifilium* and *Luffa aegyptiaca* leafy vegetables decoction as consumed on high-density lipoprotein cholesterol (HDL-C) of the diabetic rats. The result showed that baseline HDL-C was lower than the end-line HDL-C in all the treatment groups except the untreated group (negative control) that had 33.68% decreased in the HDL-C level. Percentage (51.30% and 39.63%, respectively) increases were observed in the HDL-C level of diabetic rats treated with 2ml/kg bodyweight of *G. latifilium* and *L. aegyptiaca* decoction. Also, treatment with 3ml/kg bodyweight of *G. latifilium* and *L. aegyptiaca* decoction showed 65.95% and 72.15%, respectively, increases in the HDL-C level of the diabetic rats. There was 37.81% increase in the HDL-C level of rats treated with 0.5ml/kg bodyweight of glibenclamide.

Figure 2 shows the effect of *G. latifilium* and *L. aegyptiaca* decoction on the low-density lipoprotein cholesterol (LDL-C) level of the rats. The result showed 76.27% decrease in the LDL-C level of the rats treated with 2ml/kg bodyweight of *G. latifilium* decoction. There was also 80.88% decrease in the LDL-C level of rats treated with 3ml/kg body weight of *G. latifilium* decoction. The result showed 70.13% decrease in the LDL-C level of the rats treated with 2ml/kg bodyweight of *L. aegyptiaca* decoction. There was also 77.33% decrease in the LDL-C level of rats treated with 3ml/kg body weight of *L. aegyptiaca* decoction. The result also showed 73.33% decrease in the LDL-C level of the diabetic rats treated with 0.5ml/kg bodyweight of glibenclamide, while untreated diabetic rats had 17.51% increase in the LDL-C level.

Figure 3 shows the effect of *G. latifilium* and *L. aegyptiaca* decoction as consumed on the total cholesterol (TC) level of the diabetic rats. The result showed 19.80% decrease in the TC level of diabetic rats after treatment with 2ml/kg bodyweight of *G. latifilium* decoction. There was 41.53% decrease in the TC level of the rats treated with 3ml/kg bodyweight of *G. latifilium* decoction. Also, 15.51% decrease was observed in the TC level of diabetic rats treated with 2ml/kg bodyweight of *L. aegyptiaca* decoction. Treatment with 3ml/kg bodyweight of *L. aegyptiaca* showed 41.93% in the TC level of the diabetic rats. The result also showed a percentage (41.34%) decrease in the TC level of the diabetic rats treated with 0.5ml/kg bodyweight of glibenclamide, while there was 8.28% increase in the TC level of untreated diabetic rats.

Table 6 shows the effect of *G. latifilium* and *L. aegyptiaca* decoction as consumed on the aspartate amino transferase (AST) level. There was 18.80% increase in the AST level of untreated diabetic rats. About 5.84% decrease was observed in the AST level of diabetic rats treated with 2ml/kg bodyweight of *G. latifilium* decoction. Treatment with 3ml/kg bodyweight of *G. latifilium* decoction showed 31.34% decrease in the AST level of the diabetic rats. The result also showed 18.96% decrease in the AST level of the rats after treatment with 2ml/kg bodyweight of *L. aegyptiaca* decoction, while treatment with 3ml/kg bodyweight of the same decoction showed 28.53% decrease in the AST level of the diabetic rats. There was about 23.82% decrease in the AST level treated with 0.5ml/kg bodyweight of glibenclamide.

Table 7 shows the effect of *G. latifilium* and *L. aegyptiaca* decoction as consumed on alkaline phosphate (ALP) of the diabetic rats. There was 7.45% increase in the ALP level of untreated diabetic rats. About 15.17% decrease was observed in the ALP level of diabetic rats treated with 2ml/kg bodyweight of *G. latifilium* decoction. Treatment with 3ml/kg bodyweight of *G. latifilium* decoction

showed 29.59% decrease in the ALP level of the diabetic rats. The result also showed 16.48% decrease in the ALP level of the rats after treatment with 2ml/kg bodyweight of *L. aegyptiaca* decoction, while treatment with 3ml/kg bodyweight of the same decoction showed 23.08% decrease in the ALP level of the diabetic rats. There was about 14.93% decrease in the ALP level treated with 0.5ml/kg bodyweight of glibenclamide.

Table 8 shows the effect of *G. latifilium* and *L. aegyptiaca* decoction as consumed on alanine amino transferase (ALT) of the diabetic rats. There was 10.32% increase in the ALT level of untreated diabetic rats. About 12.91% decrease was observed in the ALT level of diabetic rats treated with 2ml/kg bodyweight of *G. latifilium* decoction. Treatment with 3ml/kg bodyweight of *G. latifilium* decoction showed 20.27% decrease in the ALT level of the diabetic rats. The result also showed 21.46% decrease in the ALT level of the rats after treatment with 2ml/kg bodyweight of *L. aegyptiaca* decoction, while treatment with 3ml/kg bodyweight of the same decoction showed 19.35% decrease in the ALT level of the diabetic rats. There was about 23.98% decrease in the ALT level treated with 0.5ml/kg bodyweight of glibenclamide.

Discussion

The phytochemical constituent of *Gongronema latifilium* and *Luffa aegyptiaca* leafy vegetables decoction showed the presence of alkaloid, phenol, carotenoid and flavonoids. The result was partly similar to that of Bamisaye et al. (16) who reported the presence of carotenoid and flavonoids in aqueous extract of M. lucida, while there was no phenol identified in their extracts. Akroum (17) observed that flavonoids have antioxidant and detoxification potentials and many health promoting effects. These qualities make *Gongronema latifilium* and *Luffa aegyptiaca* leafy vegetable decoctions a very important plant in the management of diabetes and other chronic diseases.

The high level of carotenoid (689.67 mg/100mL - 936.67 mg/100mL) in the vegetables decoction could be useful to people suffering from degenerative diseases such as aging, cataracts and Hodgkins lymphoma. The high values of carotenoid obtained in the leady vegetables decoction in traditional remedies, and thus, contributes to the management and prevention of lifestyle associated diseases such as diabetes heart diseases.

The blood glucose of the different rat groups treated with different levels of the vegetables decoction decreased with a range from 61.92% - 69.64%. The decrease was comparable to that of positive control group (57.71%). However, the sustained high value (324.8 mg/dL) representing 48.58% increase in blood glucose level of the negative control group confirms the blood glucose lowering potentials of the leafy vegetables decoction. The result is in line with the study by Ugochukwu & Babaddy (18) who documented a remarkable decrease in blood glucose level from 4.44 mmol/L to 1.66 mmol/L after administration of ethanol and methanol extracts of *Gongronema latifilium* for three weeks. The decrease in blood glucose level observed in the study may be attributed to the activities of phytochemicals present in the vegetable decoctions. Consumption of alkaloids in vegetable by animals, affect glucagon thyroid stimulating hormones and inhibit enzymes activities. The high alkaloids value observed in the vegetable decoction played a role in reducing the amount of glucagon secreted by the pancreas to bring about a reduction in blood glucose level. Furthermore, several natural flavonoids are known for their multi-biological function including anti-diabetic effects (19,20). Other activities associated with flavonoids include, anti-allergic, anti-cancer, anti-inflammatory, anti-fungi, anti-viral, anti-diabetic and anti-malarial (21). The identification of flavonoid in the vegetable decoctions explain in part, why decoction reduced blood glucose level in the diabetic animal model. Also, the appreciable percentage reduction in the blood glucose level of the treated diabetic rats could be due to presence of phenol in the vegetable decoctions. Phenol inhibit carbohydrate digestion and glucose absorption in the intestine by stimulating insulin secretion from the pancreas, modulate glucose release from the liver, and thus, activate insulin receptor cells for glucose uptake in insulin sensitive tissues (22). The blood glucose lowering effect of the leafy vegetable decoctions is not likely to develop any risk of severe hypoglycemia even in prolonged therapy at a dose of 3 mL/kg bodyweight. This justifies the traditional use (as nutraceutical) of *Gongronema latifilium* and *Luffa aegyptiaca* leafy vegetables decoction in the treatment of various ailments including diabetes mellitus.

The study also showed treatment interactions on the lipid profile of both treated and non-treated diabetic rats. A treatment intervention with the leafy vegetables decoction however, normalized the indices (total cholesterol, low density lipoprotein and high density lipoprotein). The high density lipoprotein cholesterol (HDL-C) increased by 31.63% to 79.75%, across the treatment group. This is in agreement with the studies by Otamere & Aloamaka, (23) and Inbaraj & Inbaraj (24), who reported increase in the HDL-C, decrease total cholesterol (TC) and decreased low density lipoprotein cholesterol (LDL-C), following treatment with *G. latifilium* and *L. aegyptiaca* extracts. The present study is also in line with the study by Ugochukwu et al. (25) who reported a decrease in the LDL-C after treatment with *G. latifilium* extract. There was an appreciable normalization of lipid profile of the treated diabetic rats when compared with that of the negative control group. This suggests that the recovery process of the treated groups was not spontaneous, rather a gradual process. Thus, the phytochemical composition found in the vegetable decoctions is the pointer to the corrective effect of the lipid profile of the treated diabetic rats.

There was an increase in the liver enzyme activity after diabetes was induced in the rat models. This is in line with the study by Edet et al. (26) who reported increased liver enzymes after alloxan was used to induce diabetes in rats. More so, increased aspartate amino transferase (AST) and alkaline phosphatase (ALP) activities were clear manifestation of cellular leakage and loss of functional integrity of the cell usually associated with chemical diabetes mellitus. The reversal (decreased) liver enzymes activity was attributed to the leafy vegetables decoction contributing membrane stabilizing activity and thus, preventing the leakage of intracellular enzymes. This protective activity confirms previous study by Ugochukwu et al. (25) on the reduction of liver enzymes activity (AST, ALP and ALT) to near normal after administration of *G. latifilium* extracts. Similarly, Edet et al. (26) observed general decrease in the level of aspartate amino transferase (AST), alkaline phosphatase (ALP) and alanine amino transferase (ALT) activities in diabetic rats. The study lends credence to Rajkapoor et al. (27) who reported the efficacy of any hepato-protective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a hepatoxin. The results show that the leafy vegetables decoction possesses the hepato-protective potentials that stimulated reduction in the level of liver enzymes activity.

Conclusion

The study shows that phytochemicals (flavonoids, alkaloids, carotenoids, phenols) were present in *G. latifilium* and *L. aegyptiaca* leafy vegetables decoction. The phytochemical may have acted synergistically to bring about normal blood glucose level in the treated diabetic rats within three weeks of treatment. It was also observed that the treatment corrected diabetic dyslipidemia and normalized liver enzymes activity in the treated diabetic rats. It may be inferred that *G. latifilium* and *L. aegyptiaca* leafy vegetables decoction have an insulinogenic property that possibly stimulated dormant beta-cells to secrete insulin into the insulin receptor cells of the diabetic rats.

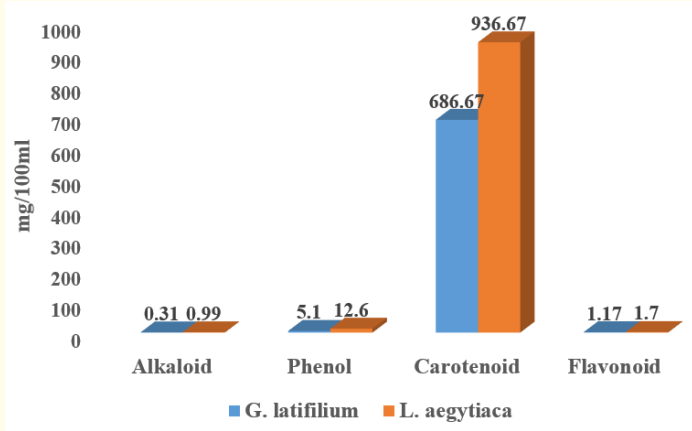

Figure 1: Phytochemical content of green leafy vegetables (*G. latifilium* and *L. aegyptiaca*) decoction as consumed.

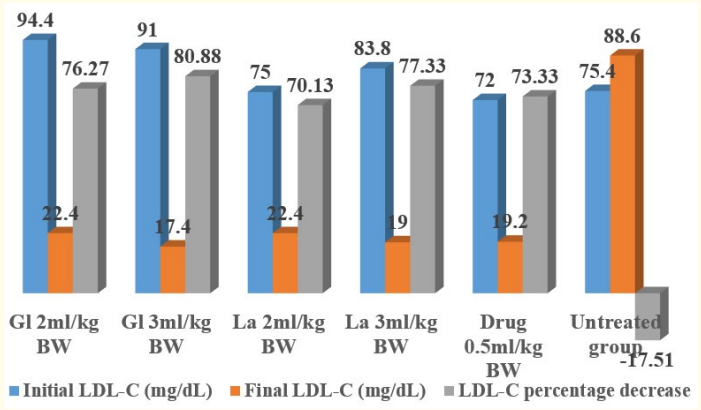

Figure 2: Low-density lipoprotein level of diabetic rats treated with green leafy vegetables (*G. latifilium* and *L. aegyptiaca*) decoction as consumed and percentage decrease.

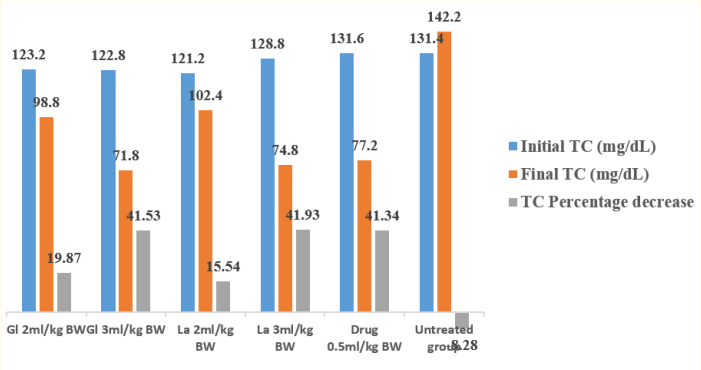

Figure 3: Total cholesterol level of diabetic rats treated with green leafy vegetables (*G. latifilium* and *L. aegyptiaca*) decoction as consumed and percentage decrease.

Groups	*Initial*	*Final*	*Mean difference*	*p-value*	*% difference*
Gl-2ml	277.4 ± 86.69	104.0 ± 4.24	173.4 ± 0.06	0.001	62.51% decrease
Gl-3ml	307.8 ± 62.27	95.8 ± 9.34	212.0 ± 0.36	0.000	68.88% decrease
La-2ml	273.6 ± 74.94	102.2 ± 8.04	171.4 ± 0.08	0.000	61.92% decrease
La-3ml	325.4 ± 52.35	98.80 ± 10.64	226.60 ± 0.35	0.002	69.64% decrease
Std drug-0.5ml	228.40 ± 43.53	96.60 ± 7.67	131.80 ± 10.17	0.000	57.71% decrease
Untreated	218.60 ± 36.49	324.80 ± 56.24	106.20 ± 10.27	0.020	48.58% increase

Gl = *G. latifilium*; La = *L. aegyptiaca*; Std = standard.

Table 4: Effect of *G. latifilium* and *L. aegytiaca* decoction on the blood glucose level of diabetics rats.

Groups	Initial	Final	Mean difference	p-value	% difference
Gl-2ml	38.60 ± 7.40	58.40 ± 9.21	19.80 ± 0.29	0.002	51.30% increase
Gl-3ml	38.80 ± 3.11	62.00 ± 5.92	23.20 ± 0.29	0.0001	59.80% increase
La-2ml	32.80 ± 3.96	45.80 ± 6.22	13.00 ± 0.23	0.001	31.63% increase
La-3ml	31.60 ± 1.67	56.80 ± 11.19	25.20 ± 0.13	0.008	79.75% increase
Std drug-0.5ml	40.20 ± 5.22	55.40 ± 4.22	15.20 ± 0.18	0.005	37.80% increase
Untreated	38.60 ± 2.41	25.60 ± 3.58	13.00 ± 0.21	0.021	33.68% decrease

Gl = *G. latifilium*; La = *L. aegyptiaca*; Std = standard.

Table 5: Effect of *G. latifilium* and *L. aegyptiaca* decoction on the high-density lipoprotein cholesterol (HDL-C) level of diabetics rats.

Groups	Initial	Final	Mean difference	p-value	% difference
Gl-2ml	58.20 ± 2.86	54.80 ± 3.11	3.40 ± 0.29	0.004	5.84% decrease
Gl-3ml	67.40 ± 5.55	46.20 ± 3.11	21.20 ± 0.08	0.010	31.34% decrease
La-2ml	53.00 ± 3.58	40.60 ± 7.38	12.40 ± 0.10	0.003	23.40% decrease
La-3ml	62.40 ± 2.07	44.60 ± 3.85	17.80 ± 0.13	0.043	28.53% decrease
Std drug-0.5ml	63.80 ± 4.15	48.60 ± 6.07	15.20 ± 0.24	0.001	23.82% decrease
Untreated	70.20 ± 7.09	83.40 ± 3.29	13.20 ± 0.37	0.020	18.80% increase

Gl = *G. latifilium*; La = *L. aegyptiaca*; Std = standard.

Table 6: Effect of *G. latifilium* and *L. aegyptiaca* decoction on the aspartate amino transferase (AST) level of diabetics rats.

Groups	Initial	Final	Mean difference	p-value	% difference
Gl-2ml	87.00 ± 2.24	73.80 ± 5.31	13.20 ± 0.15	0.002	15.17% decrease
Gl-3ml	87.20 ± 5.81	61.40 ± 6.99	25.80 ± 0.14	0.021	29.59% decrease
La-2ml	88.60 ± 6.23	74.00 ± 3.67	14.60 ± 0.23	0.004	16.48% decrease
La-3ml	83.20 ± 8.67	64.00 ± 2.74	19.20 ± 0.10	0.012	23.08% decrease
Std drug-0.5ml	84.40 ± 4.04	71.80 ± 5.93	12.60 ± 0.23	0.040	14.93% decrease
Untreated	83.20 ± 4.21	89.40 ± 2.97	6.20 ± 0.05	0.031	7.45% increase

Gl = *G. latifilium*; La = *L. aegyptiaca*; Std = standard.

Table 7: Effect of *G. latifilium* and *L. aegyptiaca* decoction on the alkaline phosphate (ALP) level of diabetics rats.

Groups	Initial	Final	Mean difference	p-value	% difference
Gl-2ml	72.80 ± 1.92	63.40 ± 3.85	9.40 ± 0.21	0.000	12.91% decrease
Gl-3ml	75.00 ± 5.48	59.80 ± 1.48	15.20 ± 0.33	0.090	20.27% decrease
La-2ml	79.20 ± 4.60	62.20 ± 3.03	17.00 ± 0.26	0.004	21.46% decrease
La-3ml	73.40 ± 3.85	59.20 ± 6.98	14.20 ± 0.11	0.010	19.35% decrease
Std drug-0.5ml	73.40 ± 3.29	55.80 ± 3.35	17.60 ± 0.27	0.040	23.98% decrease
Untreated	75.60 ± 3.51	83.40 ± 3.85	7.80 ± 0.22	0.031	10.32% increase

Gl = *G. latifilium*; La = *L. aegyptiaca*; Std = standard.

Table 8: Effect of *G. latifilium* and *L. aegyptiaca* decoction on the alanine amino transferase (ALT) level of diabetics rats.

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Conflict of interest

The authors declare that they have no conflict of interests in the study.

Author's contributions

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Conception/design of the work; interpretation of data for the work; and Final approval of the version to be published.

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Acquisition, analysis and interpretation of data for the work; Drafting of the work; Data collection; Final approval of the version to be published.

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Conception/design of the work; Acquisition, analysis and interpretation of data for the work; Drafting of the work; Final approval of the version to be published.

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