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Original Research Article

In Vivo Evaluation of Antidiabetic Effects of Some Polyherbal Formulations in Alloxan-Induced Diabetic Wistar Rats

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ABSTRACT

Diabetes is one of the most prevalent issues confronting public health in the 21st century. As humans become more interested in using plant-based formulations for the treatment of diabetes, the market for Ayurvedic, nutraceutical, and polyherbal formulations is rapidly expanding. In this study, the antidiabetic effects of polyherbal formulations of tomato, garlic, and carrot in Wistar rats are presented. The botanicals were taxonomically verified and their equal weights were shed-dried, crushed to a fine-powdered form and formulated in uniform ratios. A nutrient composition was assayed by a standard guideline. Effect of the formulations on body weight, fasting blood glucose, lipid profile, serum biochemistry, and oxidative stress were subsequently evaluated. At 150 mg/kg dose each, the formulations significantly ($p < 0.05$) improved the body weight and reduced the fasting blood glucose of the diabetic rats. There was a significant ($p < 0.05$) reduction in serum glucose, increase in haemoglobin level, restoration of the lipid profile and oxidative stress markers of the treated diabetic rats in comparison to the untreated rats. These findings have led us to the conclusion that employing safe combinations of the formulation of these botanicals for the production of antidiabetic nutraceuticals may be beneficial.

Keywords: Diabetes, Ayurvedic, Polyherbal Formulations, Tomato, Garlic, Carrot.

Introduction

Diabetes mellitus (DM) is a growing health problem distinguished by a lack of insulin production and/or insulin resistance that leads to prolonged hyperglycaemia and nutrient metabolism abnormalities.[1,2] It is one of the most prevalent issues confronting public health in the 21st century.[3] Diabetes is also one of the most difficult chronic disorders to treat in African health systems, with 19 million individuals affected in 2019.[4] With high medical costs, inaccessibility to insulin, and a variety of socioeconomic, ethnic and cultural factors, Africa accounts for the highest proportion (77%) of diabetes-related deaths worldwide.[5] In 2019, 463 million adults (20-79 years) were diagnosed with diabetes, according to the International Diabetes Federation (IDF), with the figure expected to surge to 700 million by 2045.[6]

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Diabetes can either be caused by autoimmune-mediated destruction of pancreatic β-cells, which results in entire or near-total insulin deficiency (Type 1 diabetes) or the body's inability to adequately utilize the insulin it produces for effective glucose absorption into target cells (Type 2 diabetes).[7]

Diabetes and its associated complications are now responsible for one fatality every eight seconds over the world.[2] Because of their impacts on the functions of various organs in the body; these complications are extremely important.[8] Diabetic cardiomyopathy (DCM) is a severe or chronic complication of both type 1 and type 2 diabetes. It is described as ventricular dysfunction in the absence of concomitant coronary artery disease and/or hypertension.[9] Other diabetes mellitus chronic complications include diabetic nephropathy, diabetic retinopathy, diabetic neuropathy and diabetic foot ulceration.[10-12] Diabetic ketoacidosis and hyperosmolar hyperglycaemic syndrome are some of the acute complications of diabetes mellitus.[13,14] Many metabolic disorders, including diabetes, are currently managed with the use of hypoglycaemic medications. Insulin and hypoglycaemic medicines (sulfonylureas, biguanides, and thiazolidinediones), insulin sensitizers and insulin secretagogues are the principal agents used to treat diabetes and are efficient in lowering blood glucose levels.[3] However, due to their high cost and associated side effects, these therapeutic agents' administration remains limited.[15] This prompts a quest for new treatments from a variety of sources, including traditional remedies. Medicinal plants such as *Combretum micranthum*, *Liriope spicata* and

Aloe vera[16] are being used to treat diabetes all over the world, although only a few have been scientifically validated.

Tomato (*Solanum lycopersicum*) is a savoury and edible fruit in the Solanaceae family. It is grown all over the world and eaten in a variety of ways and consumed in drinks.[17] The fruit contains a substance called lycopene, with biological credentials such as antioxidant, cardioprotective, antimicrobial and anticancer.[18] Garlic (*Allium sativum*) has been used to treat infectious diseases for millennia. For a long time, the taxonomic status of garlic has been a point of contention.[19] The ancient Egyptians utilized garlic to treat diarrhoea, and its medicinal properties were written on the walls of old temples dating to 1500 BC.[20] Carrot (*Daucus carota*) contains flavonoids, polyacetylenes, carotenoids, vitamins and minerals, all of which provide a variety of nutritional health benefits. Carrots have also been shown to have antihypertensive and hepatoprotective properties.[21]

Several experiments have demonstrated the antidiabetic effects of the individual plants; tomatoes, garlic and carrot, but no study on their cumulative antidiabetic activity has been conducted to the best of our knowledge. Studies have shown that in addition to better nutritional quality, the organoleptic qualities of polyherbal formulations can be enhanced. In addition, the pharmacological components of the herbals interact in a dynamic way to provide optimum treatment efficacy with the fewest possible side effects.[22]

Materials and Methods

Materials

Ethical clearance

Ethical approval with reference number BUK/ACUREC/21/04/C/004 was obtained from Bayero University Animal Care and use Research Ethics Committee before the commencement of the experiment.

Chemical reagents and equipment

All of the reagents and chemicals utilized in this experiment were of analytical grade and standard laboratory types of equipment were also used.

Experimental animals

In this experiment, sixty (60) Albino Wistar rats (*Rattus norvegicus*) of both sexes weighing 130 - 200 g were purchased from Bayero University Kano's Department of Human Physiology. All rats were housed in a polycarbonate cage with unrestricted access to water and pellet meal (Vital feed®, Jos, Nigeria) at a temperature of 22^{o}C and a humidity of 45-65%[23] and acclimatized for a period of two weeks (14 days). Before and during the experiment, the rats were housed in optimum conditions for animal rearing in terms of light and strict sanitation practices were put in place. The study was carried out according to the globally recognized principles for the use and care of laboratory animals, as found in the guideline of the Institute of Laboratory Animal Resources.[24]

Herbal samples

Fresh tomatoes, carrots, and garlic were purchased in the month of May, 2021, at the Ajiwa local weekly market (Latitude 12.9637^{o}N and Longitude 7.7400^{o}E) in Katsina State, North-Western Nigeria, and transported to Bayero University Kano, Nigeria. Prior to that, the samples were identified by Namadi Sunusi, a Taxonomist in the Herbarium Unit of the Department of Botany, Ahmadu Bello University Zaria. Voucher specimens numbered ABU0612 (*S. lycopersicum*), ABU12034 (*D. carota*) and ABU00423 (*A. sativum*) were archived for future reference

Methods

Samples preparation

The tomato, carrot, and garlic samples were carefully sorted out to eliminate the damaged ones. Five hundred grams (500 g) of each sample was weighed using a weighing scale to establish uniformity in their weights. The samples were shed-dried and crushed to a fine powdered form using a local grinder. The finely ground samples were transferred into numbered sealed jars and preserved under regular laboratory conditions until reconstitution was necessary. The formulation mixing ratio is shown in Table 1.

Table 1: Formulation mixing ratio

Samples	Tomato	Garlic	Carrot
A	100	-	-
B	-	100	-
C	-	-	100
D	50	50	-
E	50	-	50
F	-	50	50
G	33.4	33.3	33.3

Determination of vitamin content of tomato, garlic and carrot formulations

The level of vitamins A, C and E in the formulations was evaluated according to the standard assay protocols.[25, 26]

Mineral content analysis of tomato, garlic and carrot formulations

0.5 g of each formulation was placed in a Kjeldahl digestion flask with 24 cm^{3} of a combination of concentrated nitric acid ($HNO_{3)}$, sulphuric acid ($H_2SO_{4)}$, and perchloric acid ($HClO_{4)}$ in the ratio (9:2:1 v/v) according to the AOAC assay guideline.[27] The flask was heated after allowing it to stand overnight to prevent excess foaming. It was then digested into a clear solution before being cooled and filtered. The solution was then diluted with distilled water to the desired volume. The blank solution was made in the same way without the formulation. Using atomic absorption spectrophotometry, the mineral content (Ca, Mg, Fe, Zn, Cu, Mn, and Co) was determined. Sodium (Na) and Potassium (K) were determined using the flame photometric method, and the standards were made using sodium chloride (NaCl) and potassium chloride (KCl)

In Vivo Assay

Experimental induction of diabetes in Wistar rats

A maximum of 80 to 180 mg/kg of alloxan (20 mg interval) was tested and a minimum acceptable dose for alloxan diabetes induction was selected for the experiment to avoid the deleterious effect of a small overdosing of the diabetogenic. The chosen dose of alloxan (80 mg/kg) was delivered intraperitoneally to the rats on the final day of acclimatization, with the exclusion of those in the normal control group. To avoid alloxan-induced hypoglycaemia, rats were given a 10% glucose solution for 24 h in their cages after 6 h of alloxan administration. The rats were examined for polydipsia, polyuria, polyphagia and general bodyweight loss. The rats were starved for 24 h, and diabetes onset was confirmed using a one-touch glucometer to measure their fasting blood glucose level 72 h (3 days) following alloxan administration. Diabetic rats were included in the study if their fasting blood glucose level was greater than 7.0 mmol/L (126 mg/dL).

Dosage and reconstitution of tomato, garlic and carrot formulations

Of the corresponding formulations, 150 mg/kg body weight was administered daily (orally) as treatment using oro-gastric tube for 21 days. The grouping was designed as follows:

Group 1: Normal Control
Group 2: Diabetic Control
Group 3: Metformin
Group 4: Tomato only
Group 5: Garlic only
Group 6: Carrot only
Group 7: Tomato + Garlic (1:1)
Group 8: Tomato + Carrot (1:1)
Group 9: Garlic + Carrot (1:1)
Group 10: Tomato+ Garlic + Carrot (1:1:1)

The rats in group 1 were non-diabetic rats, non-treated; administered with a normal diet and water only. The rats in group 2 were diabetic but they were not treated while the rats in group 3 were diabetic rats treated with Metformin (100 mg/kg body weight).[28] The rats in groups 4-6 were diabetic rats treated with 150 mg/kg bodyweight of tomato,

garlic and carrot solutions respectively, while the rats in groups 7 - 10 were also diabetic and treated with 150 mg/kg bodyweight of the mixture of tomato and garlic (T:G), tomato and carrot (T:C), garlic and carrot (G:C), and the mixture of all the three (T:G:C) solutions respectively in addition to normal diet and water.

At the end of the treatment period, the animals were fasted for 12 h following the last treatment, after which they were anaesthetized by placing each rat in a plastic jar soaked with chloroform vapour. Blood was sampled from the animals via cardiac puncture into sterile labelled plastic specimen sample bottles containing EDTA for haemoglobin and glycated haemoglobin assays; the remaining blood samples were collected into plastic centrifuge tubes without anticoagulant and allowed to clot before centrifugation at 4000 rpm for 10 minutes. The serum was pipetted into labelled specimen test tubes for the estimation of biochemical parameters.

Estimation of haematological and biochemical parameters

A sensitive glucometer was used for the measurement of fasting blood glucose. The glucose oxidase/peroxidase technique was used to estimate serum glucose using a Randox kit.[29] The spectrophotometric approach was employed to estimate the total haemoglobin content of the blood samples[30] while the colourimetric assay was used for the estimation of glycated haemoglobin content.[31] The levels of total glyceride (TG), high-density lipoprotein cholesterol (HDL), and total cholesterol (TC) in serum were estimated using the instructions provided with the commercial kits. Low-density lipoprotein cholesterol (LDL) and very low-density lipoprotein cholesterol (VLDL) were estimated using the Friedewald formula.[32] The determination of α-glucosidase was done by utilizing the procedure of Matsui.[33] The procedure of Garber and Wulff[34] was employed for the quantification of serum α-amylase and serum insulin was quantified by the method of Eastham.[35]For the estimation of serum antioxidant enzymes, the malondialdehyde (MDA) level was estimated by the methods of Zubaidah.[36] The activities of reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were also assayed following their corresponding commercial kit's manuals.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Statistical differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by *Tukey's post hoc* test using Statistical Package for Social Sciences (SPSS) software version 25. A *p*-value of less than 0.05 ($p < 0.05$) was considered to be statistically significant.

Results and Discussion

Vitamin content of tomato, garlic and carrot formulations

The vitamin composition of the formulations was evaluated (Table 2). The results showed a significant difference in vitamin composition between at least two groups of formulation ($p < 0.05$). For vitamin A content, T:G:C formulation had the highest vitamin A content (19.1 mg/100mL) that was not significant ($p < 0.05$) compared to that of other formulations. Similarly, T:G:C formulation had the highest vitamin C content (34.8 mg/100mL) that was significantly ($p < 0.05$) higher compared to that of other formulations. The highest vitamin E content (1.6 mg/100mL) was also found in T:G:C formulation and it was only found to be nonsignificant ($p > 0.05$) compared to G:C formulation. Vitamins play an important role in a variety of metabolic and physiological processes.[37] Vitamin deficiencies or excesses can contribute to disease conditions by limiting normal cell growth, thus thorough and precise vitamin analysis is critical for a typical balanced diet.[38] Vitamins C and E are antioxidants, thus, making them effective therapeutic agent for the forestallment of a variety of ailments, including diabetes mellitus.[39]

Mineral content of tomato, garlic and carrot formulations

The mineral content of the different formulations is presented in Figure 1. There was an observed significant ($p < 0.05$) variation in mineral composition between at least two groups of formulations. However, a nonsignificant ($p > 0.05$) difference was observed in the composition of cobalt and zinc among all the formulations. Potassium was the most abundant element in all the formulations, followed by sodium, calcium and phosphorus. Minerals content in fruits and vegetables is also critical in meeting consumers' nutritional requirements.[40] Our findings corroborate other studies that reported the mineral composition of tomatoes, garlic and carrot.[37,41,40] When compared to the presence of other minerals, the high concentration of potassium, sodium, calcium and phosphorus in the formulations is of particular significance. Potassium is found in all cells and body fluids, and it helps to regulate heart rhythm and blood pressure in synergy with sodium while calcium is found in bones and teeth, and it helps to regulate nerve and muscle functions.[42] On the contrary, the concentration of manganese was too low in the formulations, even though, it has been conjectured that manganese may play a role in the control of glucose metabolism and kidney function.[43]

Effect of administration of different formulations on body weight changes in diabetic rats

Table 3 shows changes in bodyweight of the rats used for the experiment. Bodyweight of the diabetic rats in the diabetic control group decreased significantly ($p < 0.05$) after 21 days of diabetes induction as compared to the bodyweight of rats in the normal control and treated groups. Treatment with T:G formulation showed a noticeable gain in bodyweight close to that of the normal control group on days 7, 14 and 21 respectively. The selective extermination of the pancreatic β-cells of the islets of Langerhans by alloxan could explain the considerable drop in bodyweight of diabetic control rats compared to the treated rats. This results in continuous glucose excretion by the diabetic rats, insulin shortage, and/or insulin resistance, resulting in a decrease in peripheral glucose uptake as well as a decrease in glycogen synthesis, which could lead to structural protein breakdown, lowering an animals' body weight.[44] Consequently, the observed body weight gain in the treated groups could be due to the varied formulations' ability to lower hyperglycaemia and improve glucose metabolism, which has an antidiabetic impact. This is supported by the findings of Habibuddin[45] who studied *Caralluma sinaica* L. in streptozotocin-induced diabetic rats. The authors concluded that the plant exerted its antidiabetic effect by standing in opposition to the streptozotocin-induced glycogen exhaustion in the liver and muscle, thereby overturning the loss of weight in diabetic rabbits.

Effect of administration of different formulations on fasting blood glucose levels of diabetic rats

The effect of administration of the formulations on the fasting blood glucose of the diabetic rats is shown in Table 4.

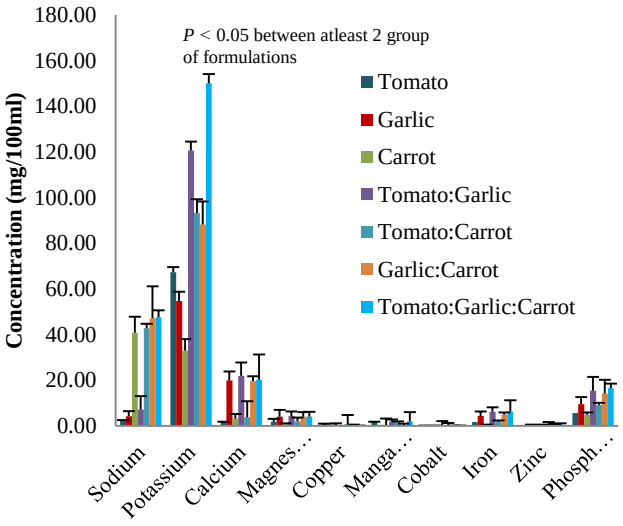

Figure 1: Mineral contents of tomato, garlic and carrot formulations

Table 2: Vitamin content of tomato, garlic and carrot formulations

	Formulation (100 g)						
Vitamin (mg/100 mL)	**T**	**G**	**C**	**T:G**	**T:C**	**G:C**	**T:G:C**
VIT. A (mg/100 mL)	4.7 ± 0.0^{a}	9.1 ± 0.1^{b}	5.3 ± 0.0^{a}	14.1 ± 0.0^{c}	9.3 ± 0.0^{b}	14.2 ± 0.0^{c}	19.1 ± 0.0^{d}
VIT. C (mg/100 mL)	10.7 ± 0.0^{a}	9.4 ± 0.2^{a}	14.5 ± 0.0^{b}	19.9 ± 0.0^{b}	26.1 ± 0.0^{c}	23.5 ± 0.0^{c}	34.8 ± 0.0^{d}
VIT. E (mg/100 mL)	0.0 ± 0.0^{a}	0.9 ± 0.0^{b}	0.7 ± 0.0^{b}	0.9 ± 0.0^{b}	0.8 ± 0.0^{b}	1.4 ± 0.0^{c}	1.6 ± 0.0^{c}

Key: **T**=Tomato only; **G**= Garlic only; **C**= Carrot only; **T:G**= Tomato:Garlic; **T:C=** Tomato:Carrot ; **G:C**= Garlic:Carrot; **T:G:C=** Tomato:Garlic:Carrot; **VIT.=** Vitamin. Mean values having different superscripts in the same row are significantly ($p < 0.05$) different

Table 3: Effect of administration of different formulations (150 mg/kg) on body weight changes in diabetic rats

	Body Weight Change (g)			
Group	**Day 0**	**Day 7**	**Day 14**	**Day 21**
NC	123.4 ± 4.0	126.1 ± 3.9^{a}	128.3 ± 4.0^{a}	128.9 ± 3.9^{a}
DC	116.3 ± 5.0	110.6 ± 5.1^{b}	101.3 ± 4.9^{b}	92.3 ± 4.6^{b}
M	117.3 ± 3.9	118.3 ± 4.0^{a}	119.4 ± 4.0^{a}	119.9 ± 4.0^{a}
T	118.8 ± 4.9	119.0 ± 6.3^{a}	122.3 ± 6.9^{a}	123.0 ± 6.3^{a}
G	116.3 ± 4.8	118.3 ± 6.3^{a}	121.3 ± 6.3^{a}	120.3 ± 3.8^{a}
C	115.9 ± 6.4	113.3 ± 7.3^{b}	110.3 ± 7.9^{a}	111.3 ± 6.8^{a}
T:G	120.3 ± 5.5	123.4 ± 4.4^{a}	125.2 ± 4.8^{a}	127.3 ± 4.3^{a}
T:C	118.3 ± 5.9	119.9 ± 4.9^{a}	122.8 ± 6.3^{a}	124.5 ± 5.4^{a}
G:C	120.3 ± 4.9	119.3 ± 5.8^{a}	120.9 ± 3.9^{a}	121.3 ± 4.0^{a}
T:G:C	121.4 ± 7.0	122.7 ± 6.3^{a}	122.9 ± 7.2^{a}	123.0 ± 7.3^{a}

Key: NC= Normal control; DC= Diabetic control; M= Metformin-treated (100 mg/kg); T= Tomato; G= Garlic; C= Carrot; T:G= Tomato:Garlic; T:C= Tomato:Carrot; G:C= Garlic:Carrot; T:G:C= Tomato:Garlic:Carrot. Values are mean ± SEM; n = 5 in each group. All experimental groups were compared to the DC in the same column. Values of significance $^{a} = p < 0.05$, $^{b} = p > 0.05$

Table 4: Effect of administration of different formulations (150 mg/kg) on fasting blood glucose levels of diabetic rats administered with different formulations (from day 0 to day 21)

	Fasting blood glucose level (mg/dL)							
Group	**Day 0**	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 15**	**Day 18**	**Day 21**
NC	75.3 ± 8.2	68.2 ± 5.4^{a}	64.3 ± 5.0^{a}	65.2 ± 5.1^{a}	66.3 ± 6.3^{a}	62.3 ± 4.71^{a}	63.8 ± 4.32^{a}	65.7 ± 5.3^{a}
DC	85.6 ± 6.0	298.2 ± 24.3^{b}	320.1 ± 28.3^{b}	343.3 ± 31.2^{b}	351.2 ± 18.3^{b}	388.4 ± 20.4^{b}	354.3 ± 23.6^{b}	353.9 ± 22.5^{b}
M	86.3 ± 4.9	310.4 ± 39.5^{b}	292.3 ± 31.8^{b}	361.3 ± 41.4^{b}	206.3 ± 21.3^{a}	146.1 ± 30.3^{a}	102.3 ± 27.8^{a}	89.3 ± 33.9^{a}
T	64.9 ± 9.3	397.2 ± 19.9^{b}	433.5 ± 16.3^{b}	400.1 ± 8.4^{b}	389.4 ± 31.7^{b}	397.1 ± 12.2^{b}	316.2 ± 12.8^{b}	289.2 ± 16.8^{a}
G	74.6 ± 5.6	299.2 ± 37.2^{b}	263.3 ± 38.6^{b}	232.5 ± 37.6^{a}	180.4 ± 32.6^{a}	163.4 ± 30.6^{a}	160.7 ± 28.3^{a}	154.3 ± 28.3^{a}
C	69.6 ± 8.3	331.4 ± 31.6^{b}	334.9 ± 38.6^{b}	330.1 ± 38.6^{b}	331.0 ± 32.4^{b}	301.6 ± 41.3^{b}	288.4 ± 39.1^{b}	260.8 ± 40.3^{a}
TG	80.9 ± 6.3	388.3 ± 20.3^{b}	300.1 ± 18.4^{b}	232.1 ± 17.1^{a}	202.2 ± 19.4^{a}	163.8 ± 21.9^{a}	121.8 ± 28.2^{a}	101.3 ± 33.8^{a}
TC	81.4 ± 5.3	431.4 ± 9.5^{b}	408.3 ± 32.2^{b}	387.3 ± 18.3^{b}	316.3 ± 21.9^{b}	286.3 ± 31.2^{b}	210.3 ± 31.6^{a}	182.5 ± 35.2^{a}
GC	87.6 ± 2.4	381.5 ± 21.8^{b}	323.4 ± 30.6^{b}	289.1 ± 18.3^{b}	206.3 ± 18.4^{a}	188.3 ± 22.9^{a}	120.3 ± 38.1^{a}	148.3 ± 86.3^{a}
TGC	83.6 ± 4.3	416.3 ± 8.4^{b}	343.3 ± 16.4^{b}	301.3 ± 21.9^{b}	261.4 ± 31.6^{b}	120.2 ± 23.9^{a}	115.3 ± 88.7^{a}	99.8 ± 36.8^{a}

Key: NC= Normal control; DC=Diabetic control; M=Metformin-treated (100 mg/kg); T=Tomato; G=Garlic; C=Carrot; T:G=Tomato:Garlic, T:C=Tomato:Carrot; G:C=Garlic:Carrot; T:G:C=Tomato:Garlic:Carrot. Values are mean ± SEM; n = 5 in each group. All experimental groups were compared to the DC in the same column. Values of significance a = $p < 0.05$, b = $p > 0.0$

The fasting blood glucose level increased after days 3, 6 and 9 of diabetes induction and began to decrease significantly ($p < 0.05$) on days 12, 15, 18 and 21 following treatment, with the T:G:C formulation-treated group having the highest reduction in fasting blood glucose level (99.8 ± 36.8 mg/dl) among the formulation-treated groups at day 21. As similarly explained by Gupta,[46] the significant ($p < 0.05$) reduction in fasting blood glucose levels of the treated diabetic rats could be attributed to the formulations ability to inhibit α-amylase activity, resulting in a drop in blood glucose levels due to monosaccharide being less available for absorption in the small intestine's mucosal border. In the treated rats, the delay in glucose absorption rate might be responsible for sustaining blood glucose levels. This finding is supported by some previous works on garlic[47], tomato[48-49] and carrot.[50]

Effect of administrations of different formulations on serum glucose, total haemoglobin and glycated haemoglobin levels of diabetic rats

Table 5. shows the effect of the different formulations on the serum glucose, total haemoglobin and glycated haemoglobin levels of the diabetic rats. The result revealed a significant variation in serum glucose level between the diabetic control (DC) and the treated groups. Among the formulation-treated groups, T:G:C formulation

had the lowest level of serum glucose (4.9 ± 1.2 mmol/l) which was highly significant compared to the DC group. There was also an observed significant ($p < 0.05$) variation among the concentrations of total haemoglobin in the experimental groups, with the T:G:C formulation-treated group having the highest total haemoglobin concentration (13.1 ± 0.1 g/dl) that was significant ($p < 0.05$) compared to the DC group. Likewise, there was an observed significant ($p < 0.05$) variation in the concentration of the glycated haemoglobin among the experimental groups in comparison to the DC group, with the T:G:C formulation treated group having the lowest glycated haemoglobin (5.1 ± 0.2 %) level among the formulation-treated groups. The significant ($p < 0.05$) reduction in serum glucose of some treated groups is related to the significant reduction in glycated haemoglobin as observed in some treated groups because the level of glycation of serum proteins is directly proportional to the level of serum glucose. Glycated haemoglobin is a distinguishing sign of diabetic patients' glycemic state. It is generated when circulating blood glucose binds non-enzymatically to haemoglobin.[51]

Effect of administration of different formulations on serum lipid profile of diabetic rats

Table 6.shows the lipid profile of diabetic rats administered with different formulations. The low-density lipoprotein cholesterol (LDL) of the diabetic rats administered with different formulations ranged from 11.0 ± 1.6 mg/dL (T:G:C) to 65.7 ± 3.6 mg/dL (DC). There was significant variation in the LDL values of the treated groups as compared to the DC group. Among the formulation treated groups, T:G:C group had the lowest value (11.0 ± 1.6 mg/dL) of LDL which was significantly ($p < 0.05$) less than that of the DC group (65.7 ± 3.6 mg/dL). Likewise, it also had the highest HDL level among the formulation treated groups that were significant ($p < 0.05$) compared to that of the DC group. There was also a significant ($p < 0.05$) variation in the total glyceride levels of the treated groups compared to that of the DC group, with the garlic formulation-treated group having the lowest total glycerides level (80.5 ± 0.6 mg/dl) among the formulation-treated groups. The concentration of the total cholesterol in the treated groups also varied significantly ($p < 0.05$) as compared to that of the DC group, with the G:C and T:G:C formulations having the lowest total cholesterol levels among the formulation-treated groups. However, no significant ($p > 0.05$) variation was observed among the very low density lipoprotein cholesterol (VLDL) values of all the groups. Lipid metabolism anomalies shown by higher levels of TC, LDL, as well as lower HDL levels, are among the complications of diabetes.[52] Furthermore, oxidative stress promotes the oxidation of low-density lipoprotein (LDL) particles in diabetes, which leads to endothelial damage and an increased risk of atherosclerosis.[53] According to Ramadan[3], diabetic dyslipidaemia is characterized by a triad of high LDL/HDL ratio, as well as hypertriglyceridaemia. Triglycerides (TGs) are normally digested by the insulin-stimulated lipoprotein lipase enzyme (LLE). In the case of diabetes, however, LLE is not triggered due to deficiency of insulin, which leads to increased TGs production by the liver and a disparity in VLDL liberation and clearance rates by LLE. As a result, TGs are frequently utilized as markers of lipid aggregation within cells.

Table 5: Effect of administration of different formulations (150 mg/kg) on serum glucose, total haemoglobin, and glycated haemoglobin levels of diabetic rats

Group	Serum glucose (mmol/L)	Total haemoglobin (g/dL)	Glycated haemoglobin (%)
NC	5.3 ± 0.3^a	12.9 ± 0.1^a	4.9 ± 0.1^a
DC	13.9 ± 0.7^b	8.5 ± 0.1^b	10.9 ± 0.1^b
M	4.1 ± 0.8^a	12.3 ± 0.4^a	5.0 ± 0.1^a
T	10.5 ± 0.4^b	9.8 ± 0.1^b	8.1 ± 0.1^b
G	6.3 ± 0.6^a	12.3 ± 0.0^a	6.0 ± 0.1^a
C	9.5 ± 0.3^a	9.0 ± 0.10^b	8.1 ± 0.1^b
T:G	5.3 ± 0.1^a	12.6 ± 0.17^a	5.7 ± 0.4^a
T:C	9.4 ± 0.3^a	9.5 ± 0.1^b	8.1 ± 0.2^b
G:C	5.7 ± 0.5^a	12.9 ± 0.1^a	6.1 ± 0.1^a
T:G:C	4.9 ± 1.2^a	13.1 ± 0.1^a	5.1 ± 0.2^a

Key: NC= Normal control, DC= Diabetic control, M= Metformin-treated (100 mg/kg), T= Tomato, G= Garlic, C= carrot, T:G= Tomato:Garlic, T:C= Tomato:Carrot, G:C= Garlic:carrot, T:G:C= Tomato:Garlic:Carrot. Values are mean ± SEM; n = 5 in each group. All experimental groups were compared to the DC in the same column. Values of significance $^a = p < 0.05$, $^b = p > 0.05$

Table 6: Effect of administration of different formulations (150 mg/kg) on serum lipid profile of diabetic rats

Group	LDL (mg/dL)	HDL (mg/dL)	TG (mg/dL)	TC (mg/dl)	VLDL (mg/dl)
NC	11.7 ± 1.2^a	44.3 ± 0.4^a	79.2 ± 0.2^a	71.7 ± 1.0^a	15.9 ± 0.0^a
DC	65.7 ± 3.6^b	20.3 ± 0.4^b	97.2 ± 0.5^b	105.4 ± 3.8^b	19.4 ± 0.1^a
M	12.0 ± 2.3^a	36.2 ± 4.0^a	80.4 ± 0.5^a	66.2 ± 2.2^a	16.1 ± 0.1^a
T	39.3 ± 3.3^a	40.7 ± 1.6^a	82.9 ± 1.1^a	96.2 ± 3.8^a	16.6 ± 0.2^a
G	21.4 ± 1.8^a	40.3 ± 0.6^a	80.5 ± 0.6^a	77.7 ± 1.9^a	16.1 ± 0.1^a
C	12.5 ± 2.1^a	42.6 ± 1.5^a	91.3 ± 0.8^a	70.6 ± 0.7^a	18.3 ± 0.2^a
T:G	20.8 ± 3.1^a	39.1 ± 1.1^a	81.2 ± 1.7^a	76.1 ± 2.1^a	16.2 ± 0.3^a
T:C	15.2 ± 1.2^a	37.6 ± 1.3^a	89.3 ± 0.8^a	70.8 ± 0.4^a	17.9 ± 0.2^a
G:C	11.2 ± 1.5^a	42.1 ± 0.9^a	83.2 ± 0.6^a	69.9 ± 1.4^a	16.7 ± 0.1^a
T:G:C	11.0 ± 1.6^a	42.8 ± 1.5^a	80.8 ± 0.3^a	69.9 ± 0.5^a	16.2 ± 0.1^a

Key: LDL= Low-density lipoprotein cholesterol; HDL= High-density lipoprotein cholesterol ;T:C= Total cholesterol; T:G=Triglyceride;T:C= cholesterol; VLDL= Very low-density lipoprotein cholesterol; NC= Normal control; DC= Diabetic control; M= Metformin-treated (100 mg/kg);T= Tomato; G= Garlic; C= Carrot, T:G= Tomato:Garlic;T:C= Tomato:Carrot; G:C= Garlic:Carrot; T:G:C= Tomato:Garlic:Carrot. Values are mean ± SEM; n = 5 in each group. All experimental groups were compared to the DC in the same column. Values of significance a = p < 0.05, b = p > 0.05

Table 7: Effect of administration of different formulations (150 mg/kg) on oxidative stress markers of diabetic rats

Group	MDA (nmol/l)	GSH (mg/dl)	SOD (mg/dl)	CAT (IU/L)
NC	56.3 ± 3.9[a]	32.3 ± 1.9[a]	25.2 ± 1.7[a]	40.3 ± 1.6[a]
DC	117.3 ± 5.3[b]	20.7 ± 2.7[b]	12.4 ± 1.1[b]	20.7 ± 2.1[b]
M	55.0 ± 2.9[a]	31.0 ± 1.6[a]	24.3 ± 1.8[a]	37.3 ± 3.0[a]
T	81.3 ± 3.9[a]	23.5 ± 2.1[b]	13.4 ± 3.0[b]	21.5 ± 1.9[b]
G	76.4 ± 2.7[a]	20.9 ± 2.4[b]	14.3 ± 1.7[b]	40.9 ± 1.3[a]
C	51.7 ± 1.4[a]	27.4 ± 2.2[a]	22.3 ± 1.3[a]	41.2± 1.3[a]
T:G	68.3 ± 2.3[a]	24.5 ± 1.9[b]	18.3 ± 2.2[a]	38.2 ± 2.3[a]
T:C	50.2 ± 2.2[a]	29.0 ± 1.9[a]	20.6 ± 1.9[a]	37.3 ± 1.8[a]
G:C	61.7 ± 1.9[a]	29.9 ± 2.2[a]	24.1 ± 1.3[a]	39.4 ± 1.9[a]
T:G:C	58.9 ± 1.7[a]	29.0 ± 1.9[a]	24.9 ± 0.9[a]	40.4 ± 2.2[a]

Key: MDA= Malondialdehyde, GSH= Reduced glutathione, SOD= Superoxide dismutase, CAT= Catalase, NC= Normal control, DC= Diabetic control, M- Metformin-treated (100 mg/kg), T= Tomato, G= Garlic, C= carrot, T:G= Tomato:Garlic, T:C= Tomato:Carrot, G:C= Garlic:Carrot, T:G:C= Tomato:Garlic:Carrot. Values are mean ± SEM; n = 5 in each group. All experimental groups were compared to the DC in the same column. Values of significance [a] = $p < 0.05$, [b] = $p > 0.05$

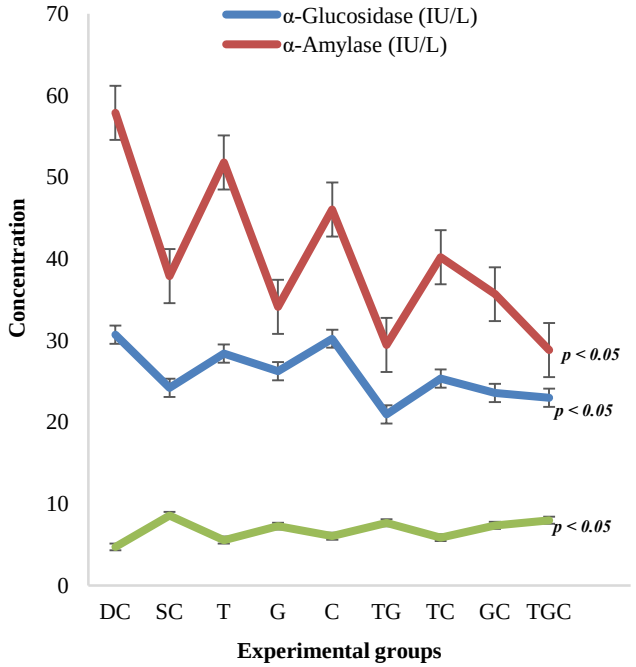

Figure 2: Effect of administration of different formulations on serum α-amylase, α-glucosidase, and insulin levels of diabetic rats.
Key: N = 6 in each group; DC = Diabetic control; SC = Standard control (Metformin treated); T = Tomato; G = Garlic; C = Carrot; TG = Tomato:garlic; TC = Tomato:carrot; GC = Garlic:carrot; TGC = Tomato:garlic:carrot;

Effect of administration of different formulations on serum α-amylase, α-glucosidase, and insulin levels of diabetic rats

Figure 2 presents the α-amylase, α-glucosidase, and insulin activities in the serum of diabetic rats treated with the different formulations. There was a significant ($p < 0.05$) difference in the level of α-glucosidase between some formulation-treated groups compared to the DC group, with the T:G formulation-treated group having the least (21.0 ± 0.4 IU/L) level of α-glucosidase that was significant compared to that of the DC group. Rats treated with T:G:C formulation showed a significant decrease ($p < 0.05$) in α-amylase (28.8 ± 2.7 IU/L) activity compared with the DC group, and even below that of the metformin-treated group (37.9 ± 0.4 IU/L). Similarly, a significant ($p< 0.05$) increase in insulin level was noted in treated groups compared to the DC group, even though, the increase was non-significant in T and T:C formulation-treated groups. The present study showed a considerable reduction in the level of α-glucosidase, with the T:G group exhibiting more of the reduction. This finding is consistence with other studies.[54,55] The reduction in blood glucose level is associated with the inhibition or lowering of α-glucosidase which decreases the absorption level of carbohydrate. The significant elevation in the serum insulin level as observed in all garlic containing formulations is also in agreement with the result of Eidi[56] who showed that garlic extract increased serum insulin in diabetic mice. However, increase in serum insulin levels is not necessarily beneficial, because induction of type-2 diabetes in animals may actually increase insulin levels due to insulin resistance

Effect of administration of different formulations on oxidative stress markers of diabetic rats

Table 7 shows the effect of administration of different formulations on the oxidative stress markers of diabetic rats. Malondialdehyde values ranged between 50.2 ± 2.2 nmol/l (T:C) to 117.3 ± 5.3 nmol/l (DC group). There was an observed significant ($p < 0.05$) variation in the malondialdehyde levels in all the treated groups compared to the DC group, with the T:C group having the lowest malondialdehyde level among the formulation treated groups. However, no significant ($p > 0.05$) difference was observed in the reduced glutathione levels in T, G and T:G formulation treated groups compared to the DC group. The superoxide dismutase level of the rats in the DC group (12.4 ± 1.1 mg/dL) showed no significant difference ($p> 0.05$) compared to that of the rats treated with tomato (13.4 ± 3.0 mg/dL) and garlic (14.3 ± 1.7 mg/dL) formulations. All other formulations showed significant increase ($p< 0.05$) in SOD level compared to the DC group. Similarly, the catalase level of rats treated with tomato formulation (T) showed no significant difference ($p > 0.05$) compared to that of the DC group. However, significant difference ($p < 0.05$) was observed in the catalase levels of the remaining treated groups compared to the DC group. Excess reactive oxygen species (ROS) and impaired antioxidant capability are thought to be major factors in the pathogenesis of diabetes.[57] It's also been reported that increased GSH and SOD levels may represent a compensatory mechanism(s) in chronic disorders like diabetes, where free radical overproduction causes oxidative stress.[58] The exhausted enzymatic (SOD and CAT) and non-enzymatic antioxidants (GSH) were significantly restored following treatment with the polyherbal formulations in our study. Malondialdehyde (MDA) is produced as a result of oxidative stress – induced lipid peroxidation. The lower MDA reductions seen in all carrot-containing formulations could be owing to the carrot's high antioxidant activity, which has been reported elsewhere.[3]

Conclusion

This study established the antidiabetic effects of the formulations of tomato, garlic and carrot in experimental diabetes induced in Wistar rats. As established in our study, T:G:C formulation exhibited better antidiabetic effect in comparison to the remaining formulations. Vitamins and minerals were found to be more abundant in the tomato:garlic:carrot (T:G:C) formulation. Similarly, diabetic rats treated with the T:G:C formulation exhibited greater improvement in body weight, reduction in fasting blood and serum glucose and increase in haemoglobin. Lipid profile (TC, TG, HDL, LDL and VLDL) and oxidative markers (MDA, SOD, GSH and CAT) of the formulation-treated rats were also restored significantly, especially in the T:G:C formulation-treated group. Most significant improvement in serum insulin by the T:G:C formulation and alleviation in the activity of α-amylase by garlic-containing formulations were as well established.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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