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Investigation of the Antidiabetic Potential of Combination of *Newbouldia leavis* (P. Beauv) Leaves and Garcinia kola (Hackel) Seed Extracts in NAD – Streptozin Induced Diabetes

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a widespread metabolic disorder characterized by insulin resistance and impaired insulin secretion, leading to hyperglycemia and various complications. Despite the availability of conventional therapies, achieving optimal glycemic control remains a challenge, necessitating alternative treatment strategies. Ethnomedicinal evidence suggests that *Newbouldia laevis* (P. Beauv) leaves and *Garcinia kola* (Hackel) seeds possess hypoglycemic properties, but their combined antidiabetic potential remains underexplored.

Objective: This study investigates the antidiabetic efficacy of *N. laevis* and *G. kola* extracts individually and in combination, aiming to develop a synergistic polyherbal formulation for diabetes management.

Methods: The study involved phytochemical screening, acute toxicity assessment, and in vivo evaluation of antidiabetic activity using a nicotinamide-streptozotocin (NAD-STZ) induced diabetic model in Swiss albino mice. The median effective doses (ED_{50}) of the individual extracts were determined, followed by an isobologram analysis to assess the interaction between the two plant extracts. Mice were divided into treatment groups receiving different combinations of *N. laevis* and *G. kola* extracts, alongside standard antidiabetic drugs as controls. Biochemical parameters, including fasting blood glucose (FBG), lipid profile, insulin secretion, and insulin resistance indices, were evaluated over 12 weeks.

Results: Phytochemical analysis confirmed the presence of bioactive compounds such as flavonoids, saponins, and terpenoids in both extracts. Acute toxicity studies showed no mortality at doses up to 5000 mg/kg, indicating safety. Both extracts significantly reduced FBG levels in a dose-dependent manner, with *G. kola* exhibiting superior glucose-lowering effects. The combination therapy demonstrated a synergistic effect, achieving enhanced glycemic control and lipid modulation compared to individual treatments. The most effective combination ratio (110 mg/kg *G. kola*: 160 mg/kg *N. laevis*) showed significant improvements in insulin sensitivity, glucose tolerance, and lipid profiles.

Conclusion: The synergistic interaction between *N. laevis* and *G. kola* offers a promising alternative for diabetes management. This combination not only improves glycemic control but also addresses metabolic complications associated with T2DM, potentially serving as a foundation for novel polyherbal formulations. Further clinical investigations are warranted to validate these findings for human application.

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Keywords: Type 2 Diabetes; Newbouldia Laevis; Garcinia Kola; Polyherbal Formulation; Insulin Resistance; Synergistic Effect

1. Introduction

Type 2 diabetes mellitus (T2DM) stands as the most widespread form of diabetes, impacting approximately 90 % of individuals with diabetes globally (1). T2DM is a persistent metabolic disorder marked by hyperglycemia, where defects in insulin secretion, action, or both, lead to elevated blood glucose levels (1, 2). Insulin, a pancreatic hormone, regulates glucose uptake and utilization in various body tissues. Insufficiency or ineffectiveness of insulin results in blood glucose accumulation, causing acute and chronic complications affecting the eyes, kidneys, nerves, heart, and blood vessels. T2DM manifests as a complex and diverse ailment influenced by numerous genetic and environmental factors that shape its risk and progression. Chief contributors to T2DM development are obesity, physical inactivity, and diet, capable of inducing or exacerbating insulin resistance and impaired insulin secretion (1). Insulin resistance occurs when tissues, particularly the liver, muscle, and adipose tissue, become less responsive to insulin, leading to diminished glucose uptake and increased glucose production.(1,2). Impaired insulin secretion arises when pancreatic beta cells fail to generate or release adequate insulin to overcome insulin resistance, resulting in elevated blood glucose levels. Various subtypes of T2DM exist, categorized by etiology, pathophysiology, and clinical presentation. Predominant insulin resistance, predominant insulin secretory defect, and mixed features are common subtypes of T2DM (3). This classification aids in diagnosing, prognosing, and treating T2DM while also revealing the underlying mechanisms and factors contributing to the disease.

The diagnosis of type 2 diabetes mellitus (T2DM) involves confirming the presence and severity of hyperglycemia, characterized by elevated blood glucose. The diagnostic landscape of T2DM is intricate and continually advancing, incorporating a blend of clinical, biochemical, and immunological criteria and methods, with variations based on individual age, ethnicity, and risk factors (4). Diagnostic criteria and methodologies for T2DM encompass the assessment of blood glucose levels, glycated hemoglobin (HbA1c), and other biomarkers reflecting the degree and duration of hyperglycemia (5). Standard methods for T2DM diagnosis include measuring blood glucose levels through fasting plasma glucose (FPG), random plasma glucose (RPG), or oral glucose tolerance test (OGTT). Glycated hemoglobin (HbA1c) offers a reliable measure of the average blood glucose level over the past two to three months and serves as a convenient diagnostic method for T2DM. Other biomarkers and tests, such as C-peptide, proinsulin, fructosamine, and continuous glucose monitoring (CGM), can provide additional insights into beta cell function and glycemic variability (4,5). The evolving landscape of T2DM diagnosis embraces the development and validation of new technologies to enhance accuracy, accessibility, and affordability. Point-of-care testing (POCT), biosensors, artificial intelligence (AI), and machine learning (ML) represent some of these innovative approaches, enabling rapid, noninvasive, and personalized diagnosis of T2DM (4,5,6)

Complications arising from type 2 diabetes mellitus (T2DM) are the negative outcomes resulting from chronic hyperglycemia (4,5). The disease is associated with eye, renal, cardiovascular, and neurological complications in the long term (7). These complications impact diverse organs and systems in the body, leading to considerable morbidity and mortality, diminished quality of life, and increased healthcare costs. Categorized as microvascular and macrovascular, these complications depend on the size and location of affected blood vessels. Microvascular complications, affecting the eyes, kidneys, and nerves, include diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy, respectively. Macrovascular complications encompass cardiovascular diseases such as coronary artery disease, ischemic stroke, and peripheral artery disease, impacting the heart and blood vessels. While the full understanding of the pathophysiology and mechanisms of T2DM complications remains elusive, factors like oxidative stress, inflammation, advanced glycation end products, polyol pathway, protein kinase C, and hexosamine pathway play roles in causing endothelial dysfunction, vascular damage, and tissue injury (8). The risk and severity of complications are influenced by the duration and extent of hyperglycemia, as well as other factors like hypertension, dyslipidemia, smoking, obesity, and genetic predisposition (4). Addressing complications of T2DM requires a challenging, comprehensive, and individualized approach, not only focusing on glycemic control but also addressing cardiovascular risk factors, comorbidities, and psychosocial aspects. The primary intervention goals are preventing or delaying complications, enhancing quality of life, and reducing mortality. Key intervention strategies involve lifestyle modification, pharmacotherapy, screening, monitoring, education, and support (4,5,6,8). However, despite the availability of various interventions and guidelines, numerous T2DM patients struggle to achieve optimal glycemic and metabolic targets, encountering barriers to accessing and adhering to care(4,8). Diabetic patients experience various vascular complications such as, atherosclerosis, diabetic nephropathy, retinopathy and neuropathy (9).

Ethnomedicinal surveys report that more than 1200 plants are being used in many ethnic societies around the world in traditional medicine for their alleged hypoglycemic activity and *Newbouldia laevis* (Bignoniaceae) is one of such plants

(10). This plant popularly known as boundary tree, chieftaincy tree, fertility plant or tree of life is locally called "Ogirisi" in Igbo, "Akoko" in Yoruba and "Aduruku" in Hausa languages. (11). All the parts of *N. laevis* have been reported to have versatile applications and are used in more than 25 medical purposes throughout the tropical Africa (12). Extracts of its root, for example, are reportedly used as traditional herbal remedies against dysentery and rheumatic swelling in the Gambia, and roundworm, migraine and ear ache in Nigeria (13). Researchers have reported many activities from this plant such as antioxidant and free radical scavenging , antimicrobial and antimalarial, hypoglycemic activities among others (10), anti-inflammatories and enzyme inhibitors (14). The plant, *Garcinia kola* Heckel is commonly known as bitter kola, false kola and male kola. It is called Akuilu in Igbo, cidagoro in Hausa and Orogbo in Yoruba languages of Nigeria (15). *Garcinia kola* is used in folklore remedies for the treatment of ailments such as liver disorders, diarrhoea, laryngitis, bronchitis and gonorrhea (16). *G. kola*, commonly known as bitter kola, has been of interest in the management of diabetes, drawing attention from researchers exploring natural alternatives for diabetes care (17,18). Several research studies have reported the potential antidiabetic properties of *G. kola* (17,18), isolation of kolaviron, a biflavonoid complex (17); 1, 3, 8, 11-benzophenones and *Garcinia* biflavanones (GB-1 GB2) and *kola* flavonone (19). We aim at investigating the antidiabetic potential of combination of leaf and seed extracts of *N. leavis* and *G.kola* respectively as well as developing a polyherbal formulation that will bail out millions all over the globe from the morbid with diabetes and its complications.

2. Materials and methods

2.1. Collection of Plant Material

Fresh leaves of *N. laevis* and seeds of *G. kola* were obtained from Ngwo, Enugu State, Nigeria and were authenticated by a taxonomist Nwafor, Felix in the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka. Voucher specimens were deposited in the herbarium of the Department of Pharmacognosy and Traditional Medicine, Nnamdi Azikiwe University, Awka with voucher specimen numbers: PCG 474/B/035 and PCG 474/G/051 respectively.

2.2. Preparation of Plant Material

The plant materials were air dried and pulverized with mechanical grinder (Gx160 Delmar 5.5HP, Honda Motor CO., LTD, Japan) into coarse powder, stored in refregirator for further use.

2.3. Animals

Adult Swiss albino rats (250 – 300 g) and mice (25 – 30 g) were used for the study. They were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Science, Nnamdi Azikiwe University, Agulu Campus. Housing of the animals was done in standard cages in the Animal House of the Department of Pharmacology and Toxicology. The animals were allowed to acclimatize for 7 days before they were used for the study. All animal experiments were conducted in compliance with NIH Guide for the Care and Use of Laboratory Animals (Pub. No. 85 – 23 Revised 1985). Ethical approval was obtained from the institution's Animal Ethical Committee.

2.4. Extraction

The dried powder of the plants (3 kg each) was extracted separately with distilled water (10 L) at 40 °C for 24 h under constant shaking. Filtration was done thereafter using muslin cloth. The filtrate was concentrated using Lyoquest Plus 55 Freeze dryer at – 40 °C. The percentage yield was determined. The dried extract was stored in a refrigerator at 4 °C

2.5. Phytochemical Analysis

The qualitative phytochemical, physicochemical and microscopic analysis were carried out using standard methods described by *Odoh et al.,* (2019) (20).

2.6. Acute Toxicity Test

The acute toxicity test was carried out using Lorke's method (21) with slight modification. The test was performed in two phases. In the first phase, 3 groups of 3 mice each were orally given 10, 100 and 1000 mg/kg body weight respectively of the extract. The animals were observed for 24 h post administration for signs of toxicity and mortality. In the second phase, 4 mice were orally administered 2000, 3000, 4000 and 5000 mg/kg body weight of the extract respectively. The animals were again observed for signs of toxicity and mortality for 24 h. The same procedure was applied for each of the extracts.

2.7. Diabetic Induction for Individual Extracts

Streptozotocin (STZ) (Sigma Aldrich, Germany) was used for the induction of diabetes in Swiss albino mice. Prior to induction, 50 mg/kg of Nicotinamide (Sigma Aldrich, Germany) was injected intraperitoneally to provide partial protection of the beta cells from complete STZ induced chemical pancreatectomy. Thereafter, Streptozotocin (100 mg/kg) was administered intraperitoneally within an interval of 15 min as described by Tahara *et al.* (2011) (22). Blood samples were drawn from the tail vein 72 h post-induction for fasting blood glucose concentration determination using One Touch Glucometer (Lifeshield, Johnson & Johnson, California). Mice with fasting blood glucose level of 160 mg/dL and above were taken to be diabetic.

2.8. Determination of Antidiabetic Median Effective Doses (Ed_{50}) of the Individual Extract

Thirty five (35) healthy Swiss albino mice each will be used for the antidiabetic median effective dose determination of both plants. They were grouped into seven groups of five mice per group. Groups1 and 2 served as controls and received 5 % Tween 20 (5 mL/kg) and metformin (100 mg/kg) p.o daily respectively. Groups 3, 4, 5, 6 and 7 were treated with daily oral administration of the plant extracts individually at doses of 50, 100, 200, 400 and 800 mg/kg respectively. Treatment was done daily via oral route for 30 days. The fasting blood glucose (FBG) levels were determined on days 0, 10, 20 and 30, and the percentage reduction in the fasting blood glucose (FBG) concentration calculated for each of the treatment groups. The 30th day blood glucose reduction of FBG produced by the extractswere used to determine the antidiabetic median effective dose (ED_{50}).

2.9. Dose Selection for Combination Study using the Isobologram Analysis

Using Loewe Additivity combination strategy (equ a/A + b/B = 1) complemented with Isobologram analysis we defined all the pairs of doses of drugs A and B that could lead to the combination effect EAB to form additivity. These were drawn a line of additivity of negative slop on a graph where the x and y axis represent the dose of drugs A and B.

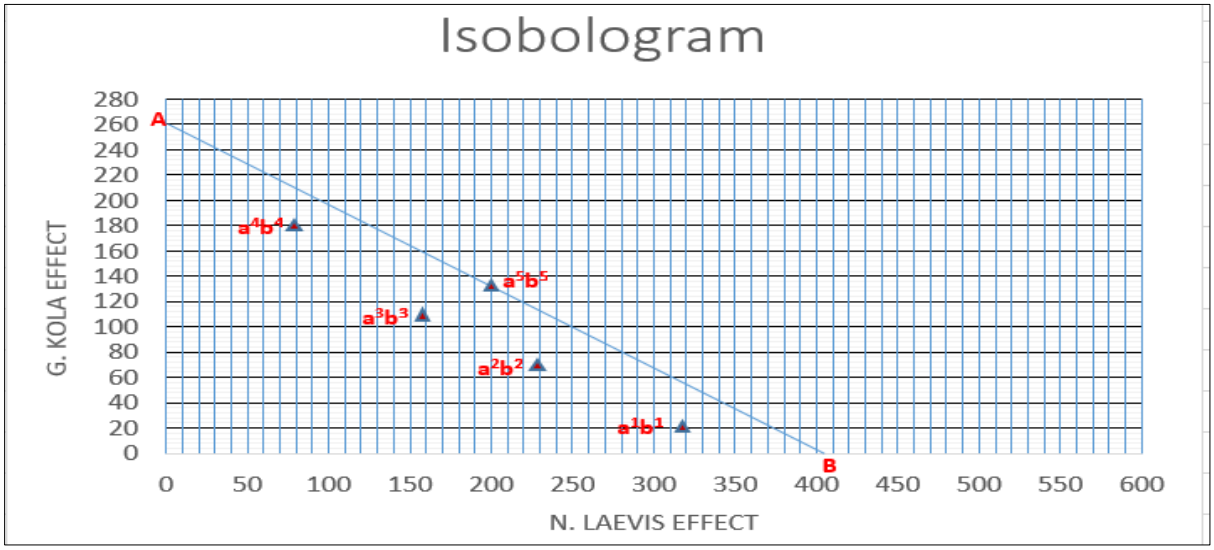

Figure 1 Isobologram: for *G. Kola* and *N. laevis*

- CI < 1 indicates synergism
- CI > 1 indicates antagonism
- CI = 1 indicates additivity

This representation made clear that when drug A is present at dose A (effective dose) the quantity of drug B needed to reach the specified level is 0. And that the presence of drug B reduced the need for drug A in a quantity predicted by the model. All experimental points below the line that could give the desired effect (50 % reduction in blood glucose) correspond to a CI < 1 and indicates synergism - these predicted synergic doses and 1 additive combination dose were selected for the study. Selected combinations were tested on STZ-NAD type 2 mice model as described previously.

2.10. Effect of Synergic Combination Doses on High-Fat Diet Streptozotocin-Nicotinamide- Induced Type 2 Diabetic Mice

2.10.1. Feed formulation

The mice were fed either a normal diet (ND) consisting (as a percentage of total calories [Kcal]) 20 % crude protein, 70 % carbohydrate, 10 % fat (total caloric energy value = 4057 Kcal/kg: Animal Care feeds, Asaba, Nigeria) ORHigh-fat (HFD) diet consisting of 20 % crude protein, 35 % carbohydrate, 45 % fat (total caloric energy value = 4057 Kcal/kg: Animal Care feeds, Asaba, Nigeria). The animals had free access to food and water and received the specified diet (Normal or High-fat) for the 12-weeks. On the day feeding began and at 3rd week, OGTT was doneto establish Pre-diabetes insulin Resistance in HFD gp prior to STZ-NAD diabetic induction.

2.10.2. Diabetic Induction for Combination Doses

At week 3, Streptozotocin (STZ) (Sigma Aldrich, Germany) was used for the induction of diabetes in Swiss albino mice. Prior to induction, 50 mg/kg of Nicotinamide (Sigma Aldrich, Germany) was injected intraperitoneally to provide partial protection of the beta cells from complete STZ induced chemical pancreatectomy. Thereafter, Streptozotocin (100 mg/kg) was administered intraperitoneally within an interval of 15 min as described by Tahara *et al*. (2011)(22). Blood samples were drawn from the tail vein 72 h post-induction for fasting blood glucose concentration determination using One Touch Glucometer (Lifeshield, Johnson & Johnson, California). Mice with fasting blood glucose level of 160 mg/dL and above was taken to be diabetic. Non diabetic mice were intraperitoneally administered with physiological saline (Henry Schein Animal Health, Dulin). Each 100 mL of the aqueous solution contained sodium chloride (0.9 g), cations {sodium: 154 meq/L}, anions {chloride: 154 mEq/L}, total osmolality is 308 milliosmoles per liter).

2.10.3. Treatment with Combination Doses

At 4 th week, Pretreatment FBG, Lipid Profile, Insulin secretion, OGTT Insulin resistance assay were carried out and treatment with combination doses began (Assay 1). Pre-treatment baseline values for blood glucose, lipid parameters and insulin secretion were established on the 5th day post diabetes induction and each of the animals received either treatment with combinations 1 and2, or standard drugs combination of glimepiride (10 mg/kg) or pioglitazone (30 mg/kg) once a day for the period of the study. Negative control groups received only the vehicle, 5 % Tween 20 (5 ml/kg). These same parameters were evaluated after 6 weeks of treatment and at the end of the study (12 weeks). The effect of the treatment on triglyceride clearance was recorded at the end of the experiment. Follow up monitoring was done at week6 for FBG determination, Lipid Profile, Insulin secretion, OGTT and Insulin resistance assays (Assay 2). At week 12, follow up monitoring was repeated (Assay 3 or Terminal assay) and this marked the end of treatment and HFD/ND feeding. The next (4th Assay) was the sub-chronic toxicity test. The effective combination ratios of *N. laevis* and *G. kola* were used for the treatment. A combination of glimepiride (10 mg/kg) and pioglitazone (30 mg/kg) were used as positive control. Negative control groups received only the vehicle, 5% Tween 20 (5 ml/kg). The following 12 groups consisting of 20 animals per group were established for the study:

- Group 1 – HFD/Diabetic with treatment 1 and 2
- Group 2 – HFD/Diabetic with Negative control
- Group 3 – HFD/Diabetic with Positive control
- Group 4 – HFD/Non-Diabetic(Prediabetes) with treatment 1and 2
- Group 5 – HFD/Non- Diabetic(Prediabetes) with Negative control
- Group 6 – HFD/Non Diabetic(Prediabetes) with Positive control
- Group 7 – ND/Non Diabetic with treatment 1 and 2
- Group 8 – ND/Non Diabetic with Negative control
- Group 9 – ND/Non-Diabetic with Positive control
- Group 10 - ND/Diabetic (STZ-oxidative stress induced) with treatment 1 and 2.
- Group 11 – ND/Diabetic (STZ-oxidative stress induced) with Negative control
- Group 12 – ND/Diabetic (STZ-oxidative stress induced) with Positive control.

Animals were weighed daily and the mean body weight determined for each group. The effect of treatment on food and water NIH consumption was also determined on a daily basis. All animal experiments were conducted in compliance with Guide for the care and use of Laboratory Animals (Pub. No. 85 – 23 Revised 1985).

At each stage of assay from baseline to sub-chronic toxicity assay, 5 mice were assayed in each group.

3. Results

Table 1 The result of the qualitative phytochemical analysis and yield

Phytocompounds	***G. kola***	***N. laevis***
Saponins	+++	+++
Cardiac glycosides	+++	+++
Tannins	++	++
Flavonoids	+++	++
Alkaloids	-	-
Steroids	+++	+++
Terpenoids	+++	+++
Yield (%)	9.17%	14.26%

+ = trace; ++ moderately abundant; +++ abundant, - = absent

3.1. Acute Toxicity Studies

Administration of both extracts up till 5000 mg/kg did not produce mortality, however; at higher doses of 4000 and 5000 mg/kg, the physical activities of the animals were reduced after the administration but normalized 2 h post administration.

3.2. Diabetic induction and Antidiabetic studies of individual extracts

The pre-induction fasting blood glucose concentration across groups were in the same range with no significance (P>0.05) differences (Figures 2,3,4 and 5). However, induction of diabetes with STZ-NAD produced a significant (P<0.05) 3-fold increase in fasting blood glucose (FBG) concentration compared to the pre-induction and naïve control group values. At the post-induction stage before treatment commenced, similar values were maintained between the vehicle control group and treatment groups. Dose dependent reduction in FBG was recorded following daily treatment with *G. kola* and *N. laevis* as a monotherapy and combination therapy. *G. kola* and *N. laevis* extracts produced significant (P<0.05) reduction in FBG from 50 mg/kg to 800 mg/kg doses from day 10 although higher reductions were achieved with *G. kola* compared to *N. laevis*. At higher doses of *G.kola*, (400 and 800 mg/kg), FBG was reduced on the 30th day below the diabetic benchmark of 160 mg/dl while *N. laevis* achieved same effect only at 800 mg/kg. Half-maximal effective doses on Day 10, 20 and 30 of treatment with *G. kola* were 25,118, 1,122 and 262 mg/kg respectively while that of *N. laevis* were 58,884, 3,548 and 404 mg/kg respectively.

Using the Loewe Additivity combination strategy (equ a/A + b/B = 1) complemented with Isobologram analysis, all the pairs of doses of drugs A (*G. kola*) and B (*N. laevis*) that could lead to the combination effect EAB to form additivity were defined. These dose pairs were drawn as a line of additivity of negative slope on a graph where the x and y-axis represent the drugs A and B doses. This representation makes clear that when drug A is present an effective dose (262 mg/kg) the quantity of drug B needed to reach the specified level (50 % reduction in blood glucose) was 0, and that the presence of drug B reduced the need for drug A in a quantity predicted by the model. All experimental points below the line that could give the desired effect correspond to a CI < 1 and indicate synergism. The following experimental points below the line of additivity were tested 20 mg/kg *G. kola*: 320 mg/kg *N. laevis* (a1ba), 70 mg/kg *G. kola*: 240 mg/kg *N. laevis* (a2b2), 110 mg/kg *G. kola*: 160 mg/kg *N. laevis* (a3b3), 180 mg/kg *G. kola*: 80 mg/kg *N. laevis* (a4b4). Also one experimental point on the line of additivity was added to the experimental protocol (131 mg/kg *G. kola*: 202 mg/kg *N. laevis* (a5b5)). These combination doses produced significant (P<0.05) reduction in FBG from day 10 while a3b3 and a5b5 where the only combination doses that reduced FBG below 160 mg/dl on the 30th day. These two dose combinations in addition to a2b2 combination dose produced up to 50 % reduction in FBG which was the set therapeutic target. Since a5b5 combination dose produced additive interaction with combination index = 1, the other two combination doses (a2b2 and a3ba) with combination indices < 1 were selected for further studies on high fat diet STZ-NAD induced type 2 diabetes.

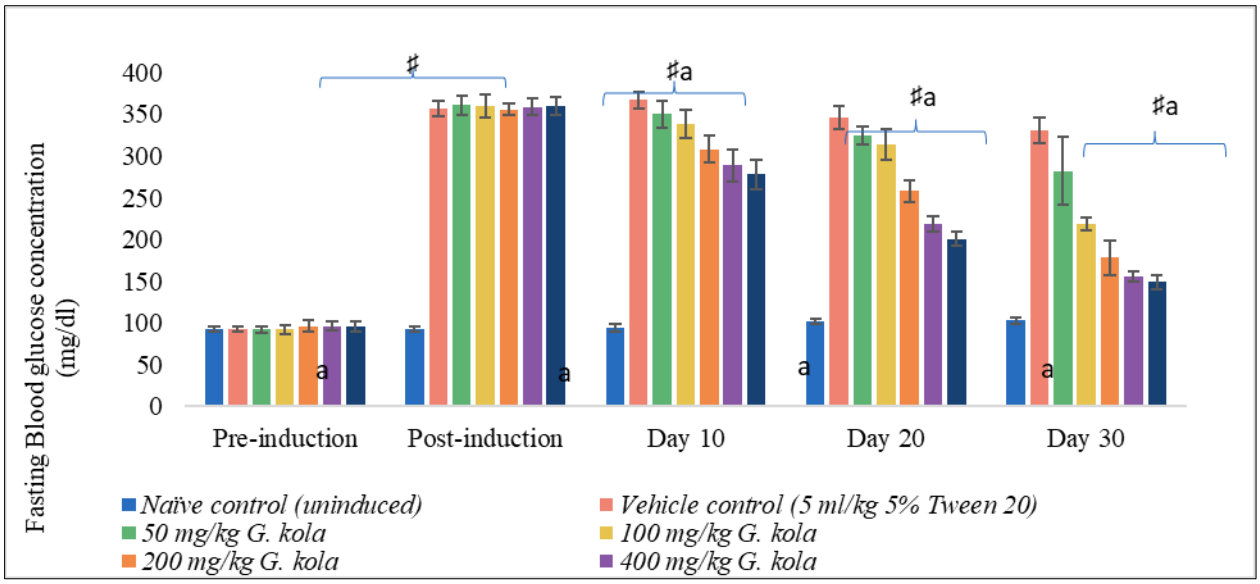

Figure 2 Repeated dose effect of *G. kola* on blood glucose concentration in STZ-NAD Induced type 2 diabetes

Where ♯= *P*<0.05 compared to pre-induction values; a = *P*<0.05 compared to vehicle control

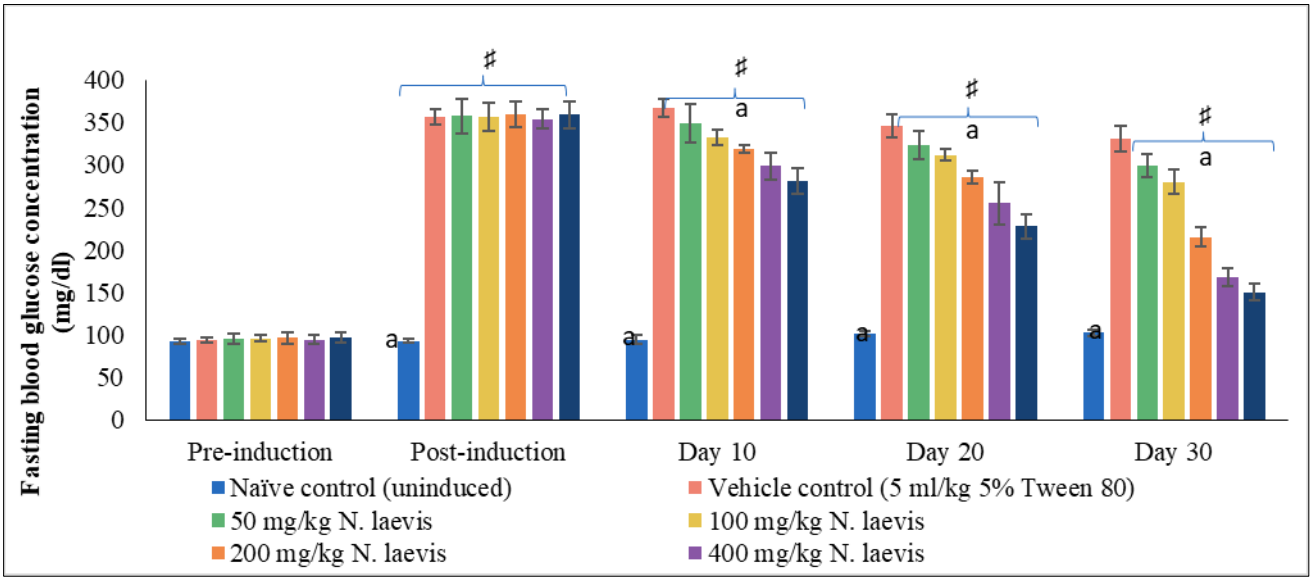

Figure 3 Repeated dose effect of *N. laevis* on blood glucose concentration in STZ-NAD induced type 2 diabetes

Where ♯= *P*<0.05 compared to pre-induction values; a = *P*<0.05 compared to vehicle control

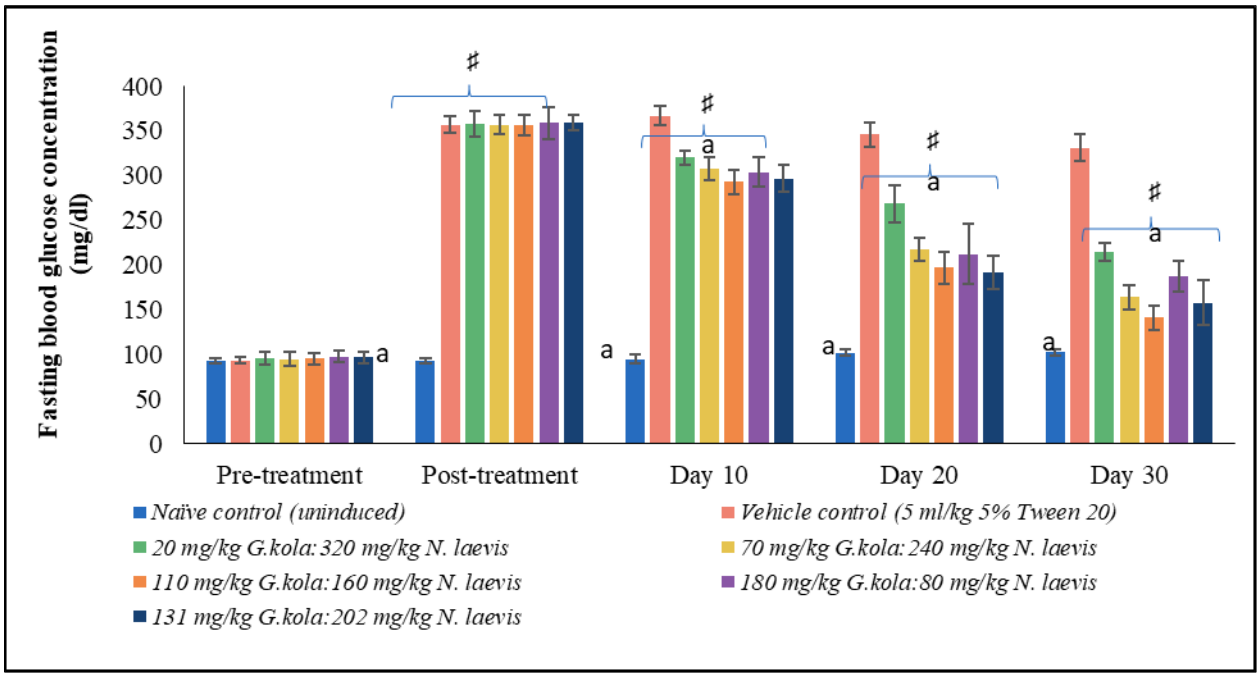

Figure 4 Repeated dose effect of *G. kola* and *N. laevis* on blood glucose concentration in STZ-NAD induced type 2 diabetes

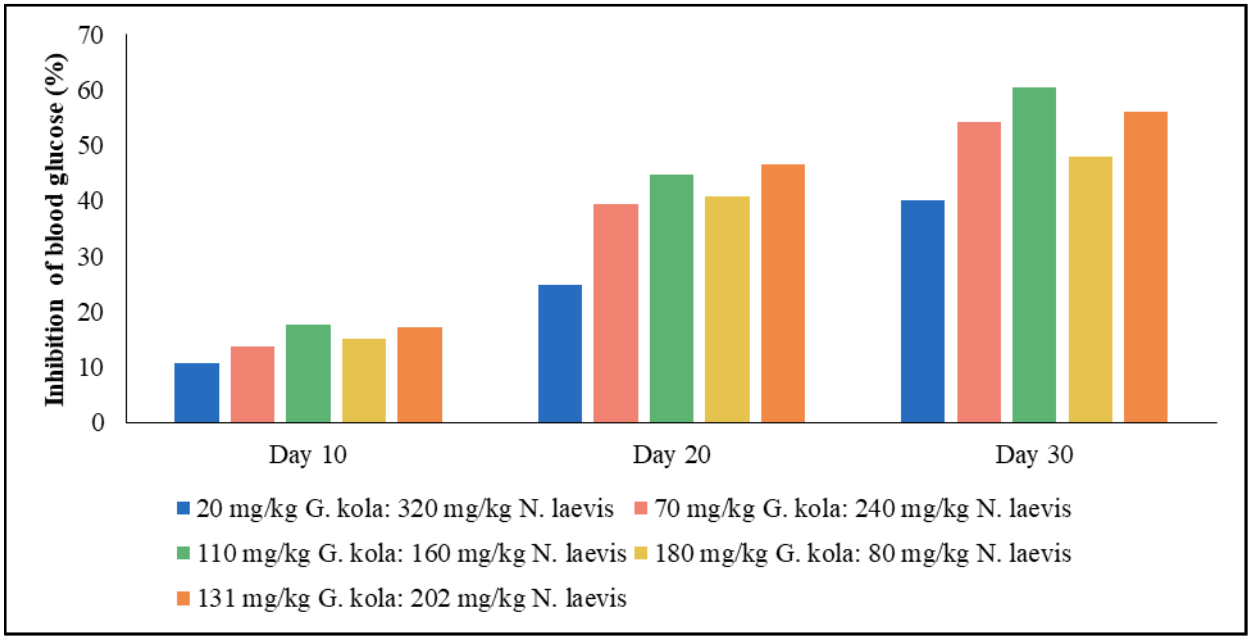

Figure 5 Repeated dose effect of combined doses of *G. kola* and *N. laevis* on blood glucose concentration in STZ-NAD induced type 2 diabetes

Where ♯= $P<0.05$ compared to pre-induction values; a = $P<0.05$ compared to vehicle control

3.3. Effect of synergistic combination doses on high-fat diet (HFD) streptozotocin-nicotinamide-induced type 2 diabetic mice

3.3.1. Effects on body weight

All the groups showed a progressive increase in weight till the 5th week. Treatment with the extracts caused a significant (P<0.05) reduction in body weight (Figures 6,7,8 and 9 and figures 14,15,16 and 17) compared with the negative control group. Diabetic animals fed with HFD generally had an increase in weight more than the diabetic group fed with ND. Between 5 th and 8 th weeks, the Gil+HFD+D group showed a significant increase (P<0.05) in weight compared with others in the group. In the 12th week, there was a significant difference in weight in all the groups in HFD +D compared with the vehicle. The HFD +NOD and the ND+NOD groups maintained a progressive increase in body weight throughout the study although with higher values observed in the HFD+ NOD group. There was a persistent increase in body weight in both HFD+NOD and ND + NOD groups though with higher values in the HFD +NOD group till the end of the study at the 12th week. The HFD +NOD group showed a significant (P<0.05) reduction in weight of the 110G: 160N +HFD+NOD group compared with the vehicle +HDF+NOD group.

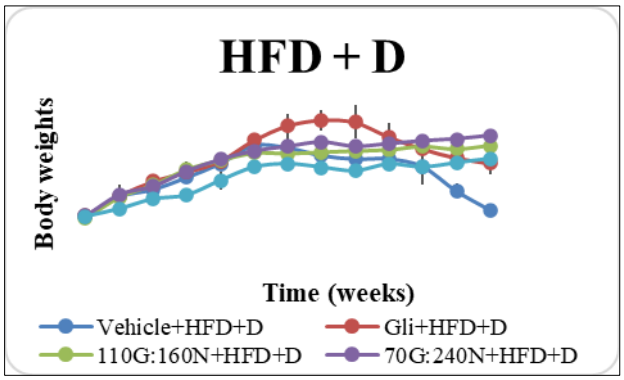

Figure 6 Effect of Treatment on body weight

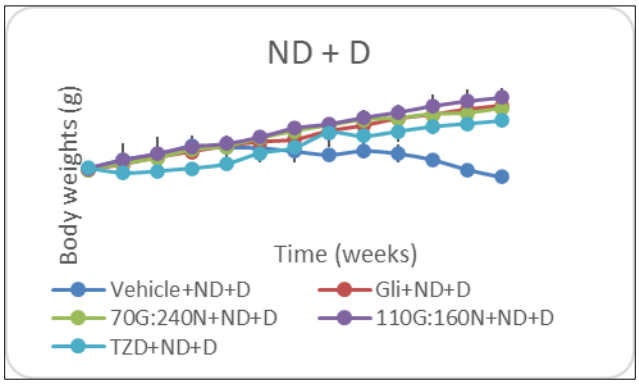

Figure 7 Effect of Treatment on body weight

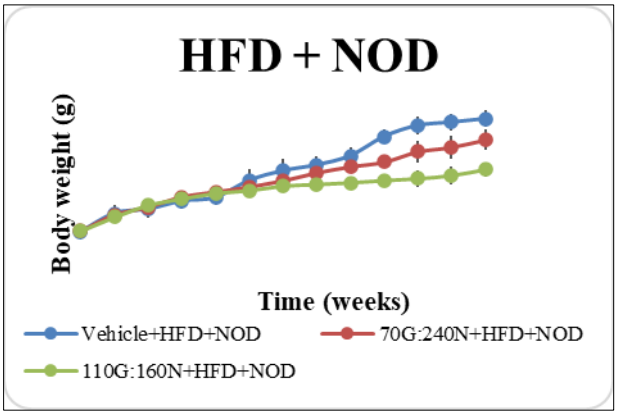

Figure 8 Effect of Treatment on body weight

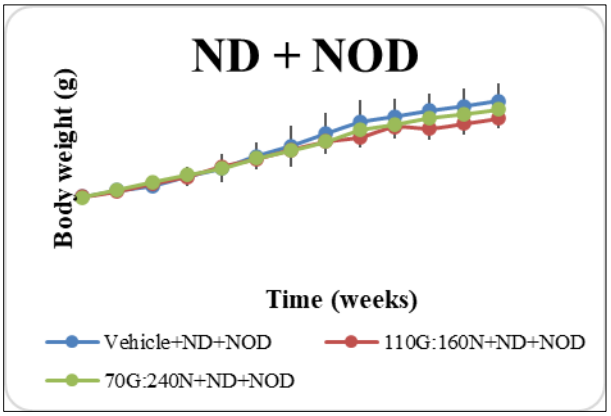

Figure 9 Effect of Treatment on body weight

3.3.2. Effects on feed consumption

On the feed consumption, the HDF +D and ND+ D dose groups showed a reduction of feed consumption from the 3rd week following treatment (Figures 10,11,12 and 13, and Figures 18,19,20 and 21). Generally both groups showed a progressive increase in feed consumption from 1st week but showed a decline from the 3rd week after treatment. The diabetic mice in the HDF +D and ND+D groups showed peak consumption in different weeks. In the HDF+ D group, peak consumption for the 70 G: 240 N subgroup was on the 2 nd week, the 110 G: 160 N group was on the 3 rd week, while the vehicle +HFD+D group was on the 5 th week. The positive control groups Gli+HFD+ D and TZD+HFD+D showed peak consumptions at the 5 th and 6 th weeks respectively, and afterwards showed a continual decrease in feed consumption. Generally, the HFD +NOD group showed a higher feed consumption than the ND+NOD group. All the animals in the

HFD+NOD maintained a continuous increase in feed consumption until the 4th week, afterwards the combination-treated groups showed a significant (P<0.05) decline in feed consumption compared with the vehicle group.

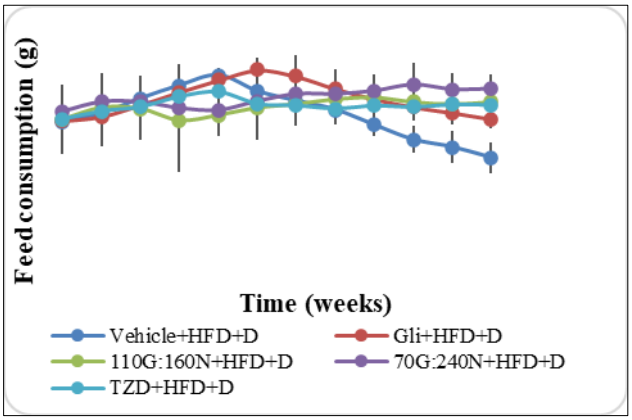

Figure 10 Effect on feed consumption

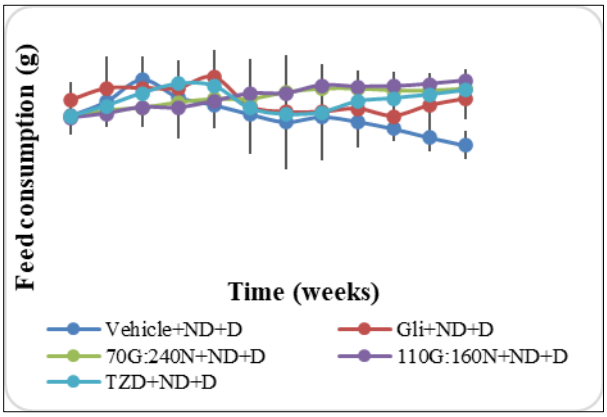

Figure 11 Effect on feed consumption

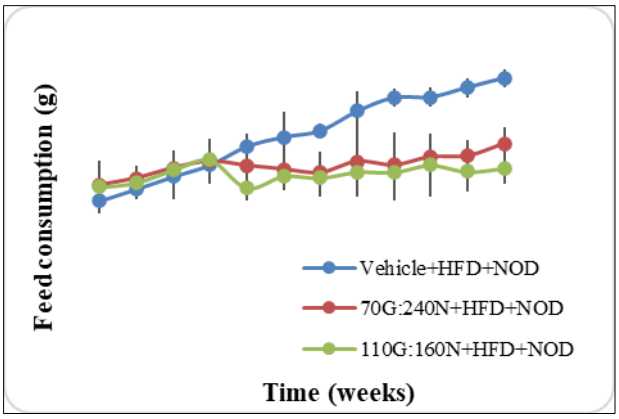

Figure 12 Effect on feed consumption

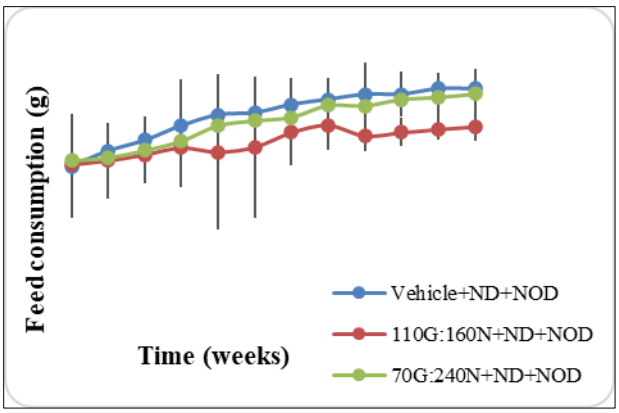

Figure 13 Effect on feed consumption

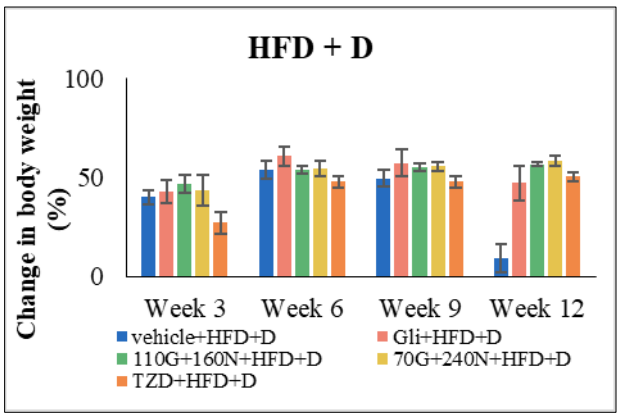

Figure 14 Change in body weight

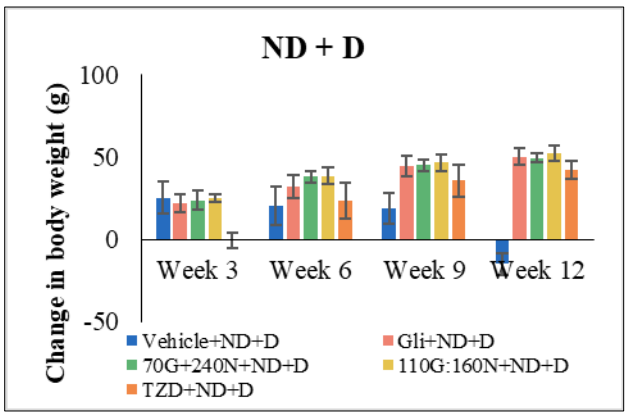

Figure 15 Change in body weight

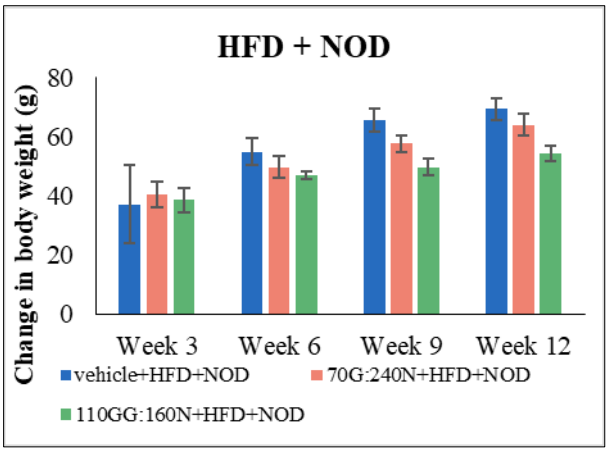

Figure 16 Change in body weight

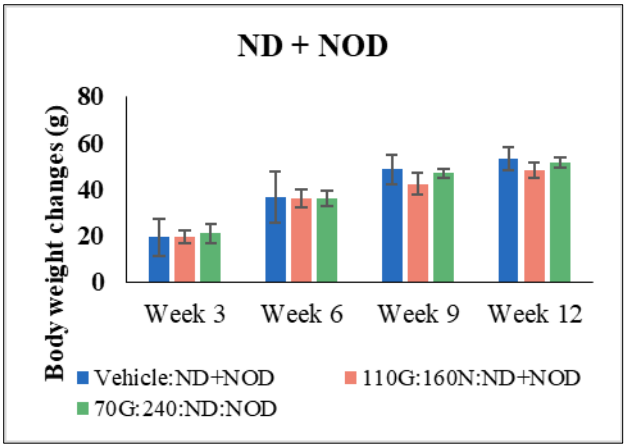

Figure 17 Change in body weight

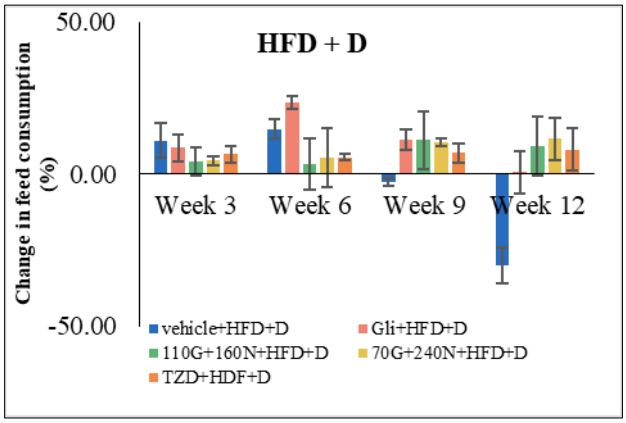

Figure 18 Percentage change in feed consumption

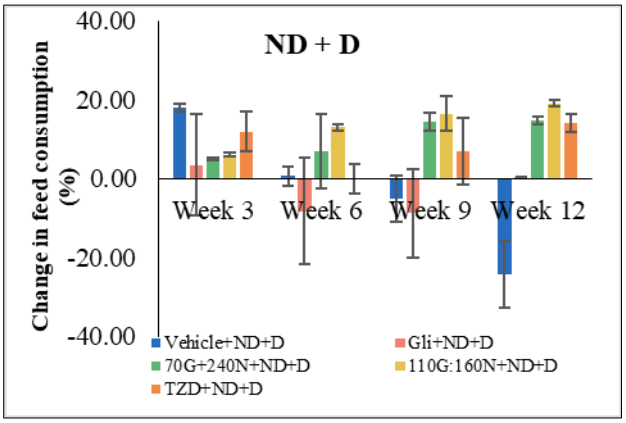

Figure 19 Percentage change in feed consumption

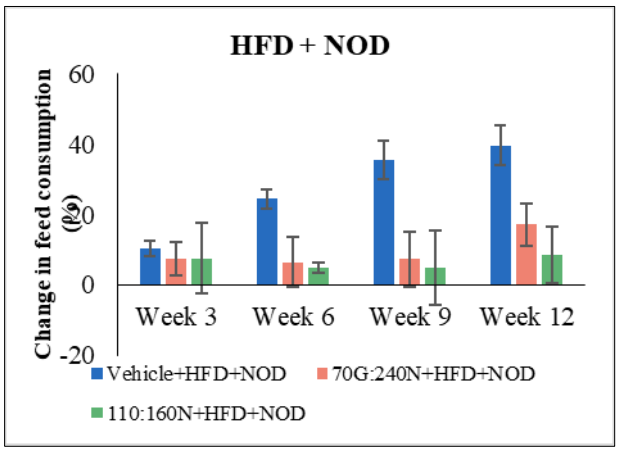

Figure 20 Percentage change in feed consumption

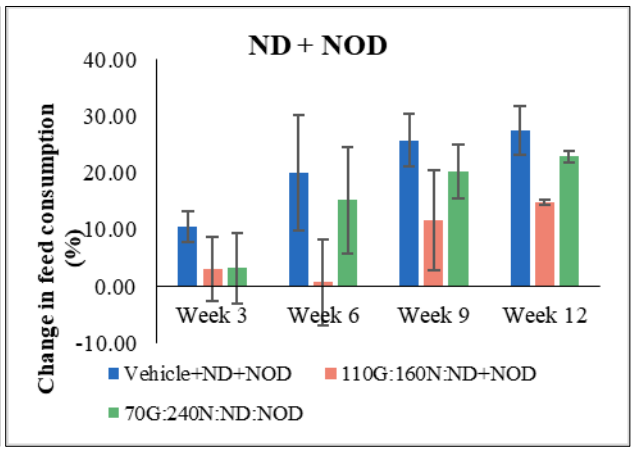

Figure 21 Percentage change in feed consumption

3.3.3. Oral glucose tolerance

In experimental diabetic vehicle control animals on high fat diet, there was progressive intolerance to oral glucose (Figures 22,23,24 and 25) as demonstrated by increase in area under the glucose time curve (AUC). Compared to the pre-treatment/basal value, the vehicle control group produced significant (P<0.05) increase from the 6 th week. Treatment with glibenclamide was unable to improve oral glucose tolerance in these high fat diet fed diabetic animals as AUC values in this treatment group was similar to that of the vehicle control group without any significant differences between these two groups on the 6th week of treatment. As treatment progresses, glibenclamide on the 12 th week

showed significant (P<0.05) reduction in AUC when compared to vehicle control group, although still maintained significantly (P<0.05) higher value when compared to its pre-treatment value. However, *G. kola* and *N. laevis* extract at the tested combination ratios were able to counter high fat diet-diabetes induced glucose intolerance. These combination treatments exhibited significant (P<0.05) decrease in AUC from the 6th week when compared to pre-treatment values as well as when compared to both glibenclamide and vehicle control groups. Pioglitazone also showed significant (P<0.05) reduction in AUC which was superior to other treatments. For normal diet diabetic experimental condition, vehicle control group still maintained significant (P<0.05) increase in AUC from the 6th week although with lower magnitude compared to the increase recorded in high fat diet diabetic experimental condition. Pioglitazone just as in high-fat diet diabetes experimental condition also produced a significant (P<0.05) reduction in AUC in the normal diet diabetic experimental condition from the 6th week when compared to the pre-treatment value and vehicle control group. Treatment of the diabetic animalson a normal diet with glibenclamide showed improved oral glucose tolerance with a significant reduction in AUC on the 6th week which was also significantly (P<0.05) better than the reduction produced by pioglitazone on the 6 th week. The effect produced by 110 mg/kg *G. kola*: 160 mg/kg *N. laevis* combination ratio in the 6 th week was also not significant (P<0.05) different from that produced by pioglitazone. Compared to the pre-treatment values and vehicle control group, all the treatments produced significant (P<0.05) reduction in AUC on the 12th week. The effect produced by 110 mg/kg *G. kola*: 160 mg/kg *N. laevis* combination ratio was similar to that produced by glibenclamide on the 12 th week without significant (P>0.05) differences between these groups. Pioglitazone also maintained a superior effect when compared to group other treatments on week 12. In both high fat diet non-diabetic and normal diet non diabetic experimental conditions, progressive significant (P<0.05) increase in AUC were observed for the vehicle control group from the 6 th week compared to basal values. The combination treatment at both doses significantly (P<0.05) improved glucose tolerance in these experimental conditions from week 6 compared to both pre-treatment values and vehicle control.

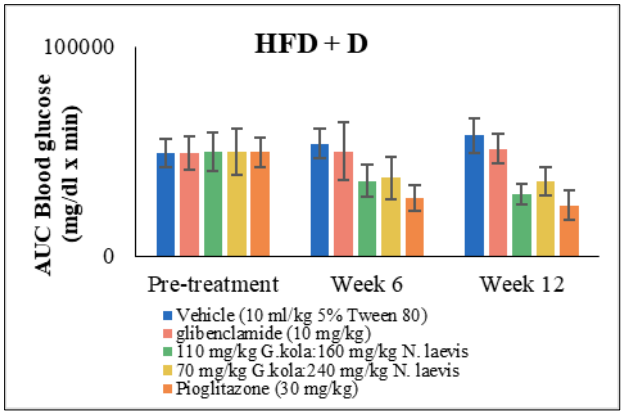

Figure 22 Oral glucose Tolerance Test (OGTT)

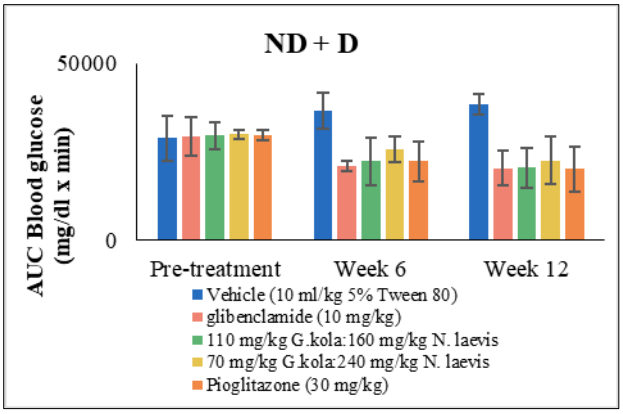

Figure 23 Oral glucose Tolerance Test (OGTT)

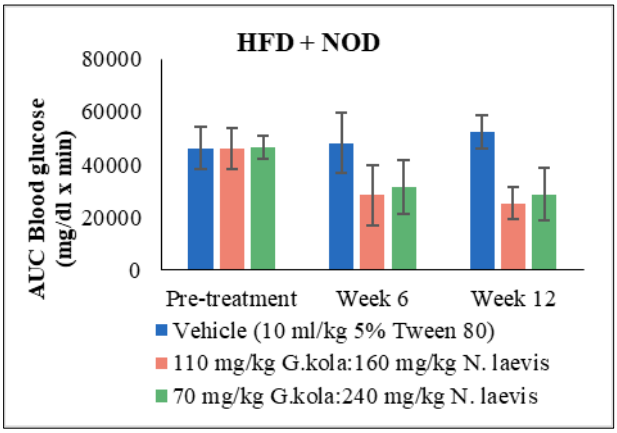

Figure 24 Oral glucose Tolerance Test (OGTT)

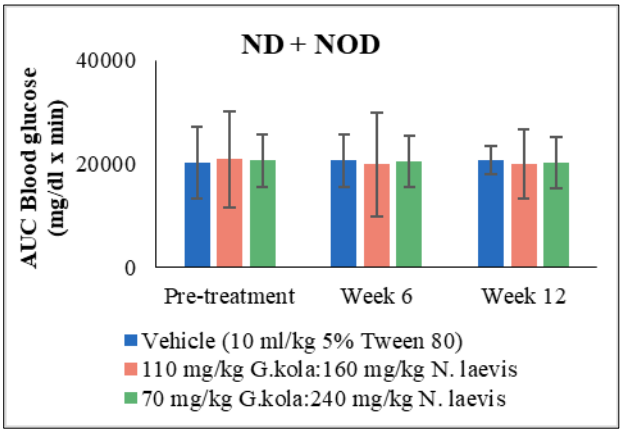

Figure 25 Oral glucose Tolerance Test (OGTT)

3.3.4. Plasma insulin and insulin resistance

Diabetic vehicle control animals on high-fat diet showed an increase in plasma insulin (Figures 26,27,28 and 29) which reached a significant (P<0.05) level in the 12th week. However, significant (P<0.05) resistance to insulin signals was recorded earlier in the 6th week (Figures 30,31,32 and 33). A similar increase in plasma insulin concentration was also recorded in the experimental group treated with glibenclamide just like in the vehicle control group. The glibenclamide treated group also showed a significant (P<0.05) increase in insulin resistance indicated by higher HOMAR-IR (Homeostatic Model Assessment for Insulin Resistance) value from the 6th week. Unlike the increase in insulin concentration in the vehicle control group, a combination of *G. kola* and *N. laevis* extracts in the tested dose ratios produced a significant (P<0.05) reduction in insulin concentration from the 6th week when compared to pre-treatment concentration and the vehicle control group. Similarly, the reduction in insulin concentration in the combination treatment groups was associated with a significant (P<0.05) reduction in insulin resistance. The combined doses' effects were closely related to the effect produced by pioglitazone without significant (P>0.05) differences occurring in these treatment groups. In the diabetic animals on a normal diet, no significant (P>0.05) reduction in plasma insulin was observed in both the control vehicle group and treatment groups except significant (P<0.05) increase recorded in the glibenclamide treated group on the 12 th week. However, this increase was not due to insulin resistance as no significant (P>0.05) changes was recorded across groups in HOMA-IR value when compared to both pre-treatment and vehicle control group. Similarly, no significant (P>0.05) differences were recorded in both plasma insulin concentration and HOMA-IR across groups in the non-diabetic animals on normal diet.

Non-diabetic vehicle control animals on high fat diet also showed significant (P<0.05) increase in insulin resistance on the 6 th week that manifested in significant (P<0.05) increase in plasma insulin concentration on the 12 th week. Compared to the vehicle control group, treatment with the combination doses produced significant reduction in insulin resistance from the 6 th week. The reduction produced by 110 mg/kg *G. kola*: 160 mg/kg *N. laevis* combination ratio on the 12 th week was significantly (P<0.05) lower that the pre-treatment value. This combination dose ratio also showed significant (P<0.05) reduction in plasma insulin concentration on the 6 th week compared to vehicle control group while both dose ratios (110 mg/kg *G. kola:* 160 mg/kg *N. laevis* and 70 mg/kg *G. kola*: 240 mg/kg *N. laevis*) showed significant (P<0.05) reduction in plasma insulin on week 12 compared to both pre-treatment values and vehicle control value.

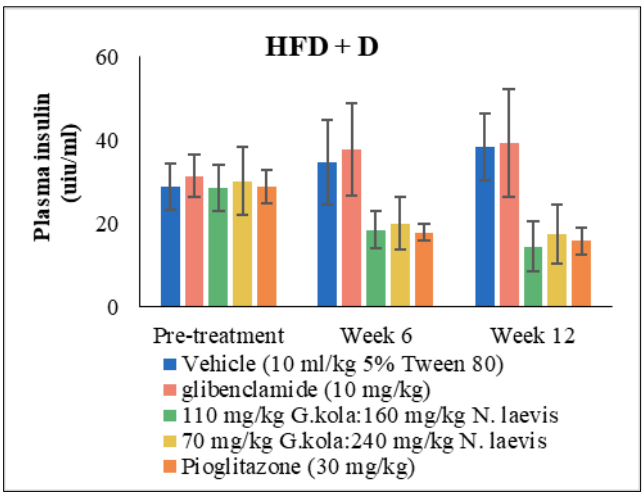

Figure 26 Insulin secretion test

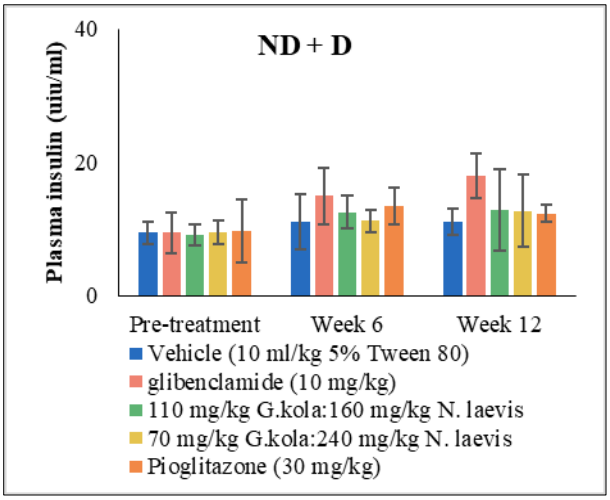

Figure 27 Insulin secretion test

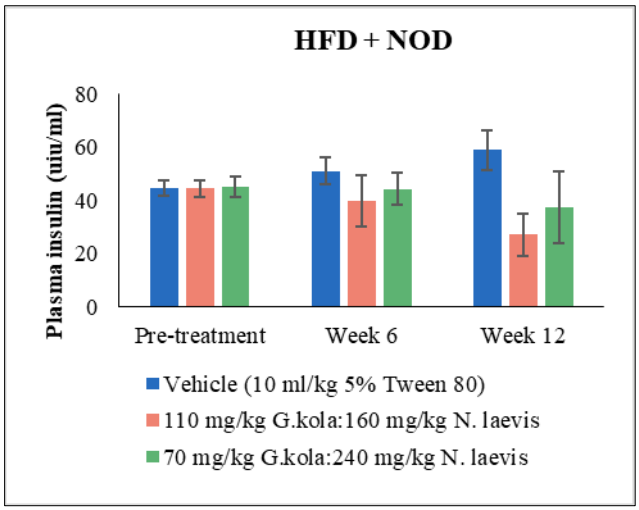

Figure 28 Insulin secretion testFigure

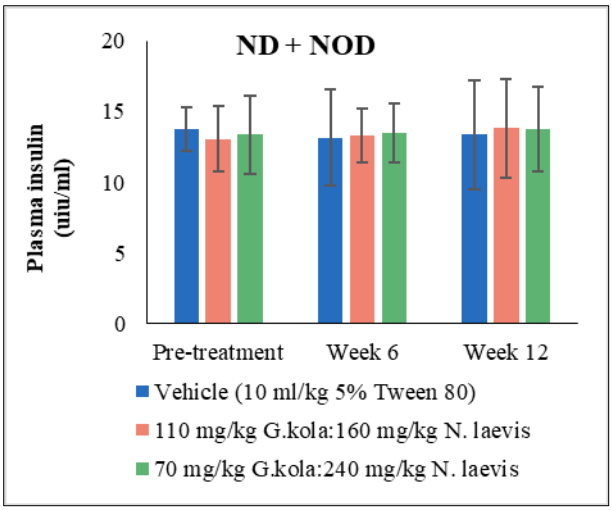

Figure 29 Insulin secretion test

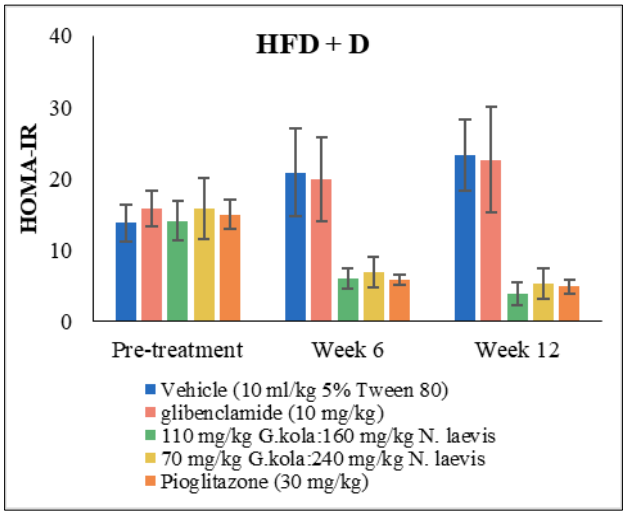

Figure 30 Effect on Insulin Resistance

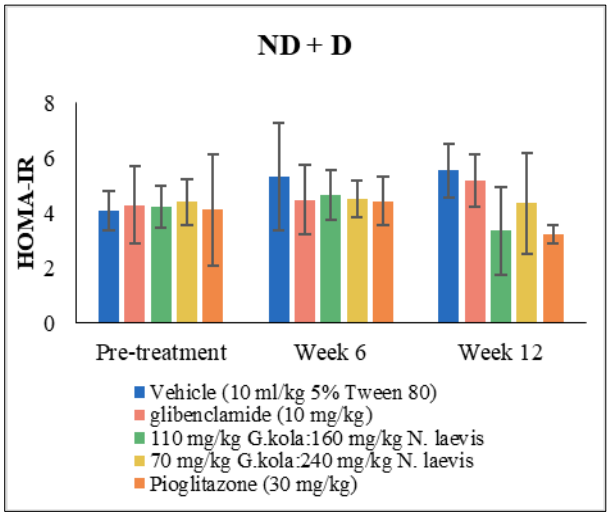

Figure 31 Effect on Insulin Resistance

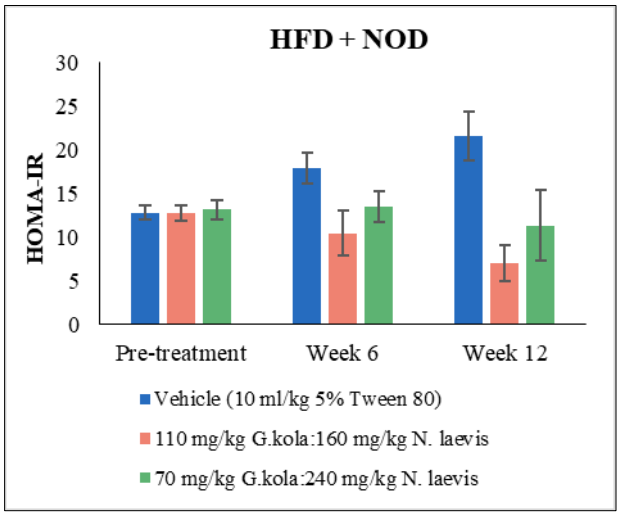

Figure 32 Effect on Insulin Resistance

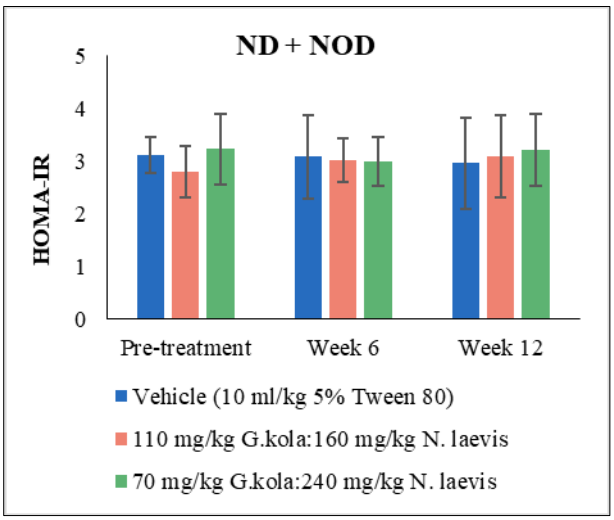

Figure 33 Effect on Insulin Resistance

3.4. Lipid profile

3.4.1. Cholesterol

Total cholesterol concentration in experimental animals placed on high fat diet (Figures 34,35,36 and 37) were generally higher (above 100 mg/dL) than animals on normal diet (below 90 mg/dL). Diabetes induction to animals on high fat diet did not show further significant (P>0.05) increase in cholesterol concentration on the 6 th week till the 12 th week when significant (P<0.05) increase in cholesterol concentration was observed in the vehicle control group compared to the pre-treatment / basal value. Animals treated with glibenclamide similarly exhibited significant (P<0.05) increase in total cholesterol when compared to pre-treatment value. Compared to the vehicle control group on the 12 th week glibenclamide treatment showed significant (P<0.05) increase in total cholesterol concentration. Conversely, treatment with pioglitazone just like with the combination doses of *G. kola* and *N. laevis* showed significant (P<0.05) reduction in total cholesterol on the 12 th week when compared to both glibenclamide and vehicle control. Also when compared to pre-treatment values, no significant (P>0.05) increase in total cholesterol was recorded for these treatment groups. The combination dose ratio - 110 mg/kg *G. kola*: 160 mg/kg *N. laevis*showed comparable effect with pioglitazone as no significant (P>0.05) difference existed between these treatment groups. Diabetes alone was able to induce significant (P<0.05) increase in total cholesterol on week 12 compared to pre-treatment/basal control value. Treatment with glibenclamide and pioglitazone produced significant (P<0.05) reduction in total cholesterol in this experimental group (ND + D) from the 6th week compared to vehicle control group. However, the combination doses were able to initiate significant (P<0.05) reduction on the 12 th week compared to vehicle control group. Pioglitazone showed similar (non-significant (P>0.05) different) effect with the combination dose ratio 110 mg/kg *G. kola*: 160 mg/kg *N. laevis* on the 12 th week. Compared to glibenclamide, these two experimental groups showed superior effect on the 12 th week. Experimental non-diabetic vehicle control animals placed on high fat diet (HFD + NOD) showed significant increase in total cholesterol from the 6th week when compared to pre-treatment/basal value. Treatment of these animals on HFD + NOD group with the combination dose of *G. kola* and *N. laevis* in the ratio of 110 mg/kg *G. kola*: 160 mg/kg *N. laevis* significantly (P<0.05) inhibited high fat diet induced increase in cholesterol on the 6th and 12th week of blood sampling compared to vehicle control group. When compared to pre-treatment/basal value, this combination dose ratio was able to exhibit significant (P<0.05) reduction in total cholesterol on the 12 th week just like 70 mg/kg *G. kola*: 240 mg/kg *N. laevis* combination dose ratio. Compared to both pre-treatment value and vehicle control group, combination dose ratio 70 mg/kg *G. kola*: 240 mg/kg *N. laevis* was unable to show significant (P>0.05) reduction in total cholesterol at the 6th week. Control non-diabetic animals placed on normal diet did not show significant (P>0.05) increase in total cholesterol over the 12 weeks' period the experiment lasted. Slight non-significant (P>0.05) reduction was recorded on the 6 th week for animals on this experimental group (normal diet non-diabetic – ND+NOD) that received the combination doses of *G. kola* and *N. laevis*. The reduction in total cholesterol concentration however, became significant (P<0.05) for these animals on combination extract treatment on the 12 th week when compared to their pre-treatment values as well as with vehicle control group.

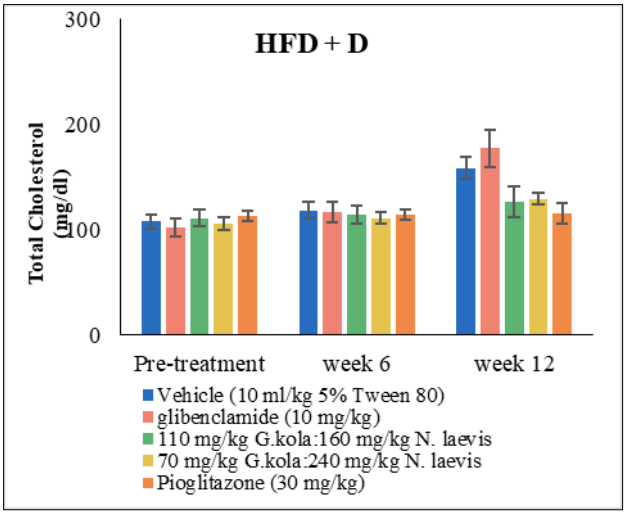

Figure 34 Effect on Total Cholesterol

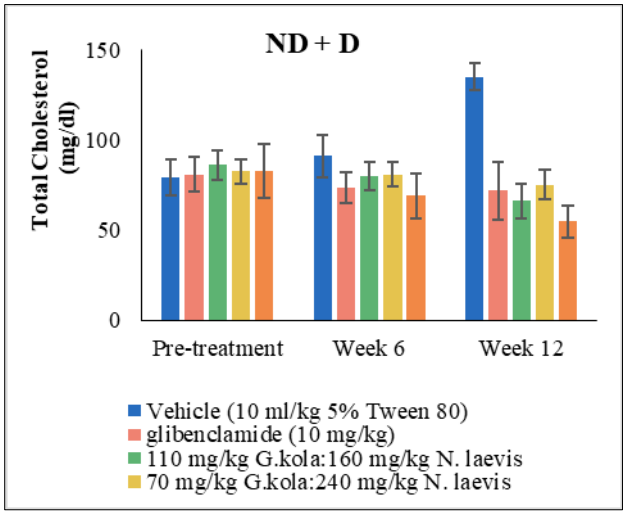

Figure 35 Effect on Total Cholesterol

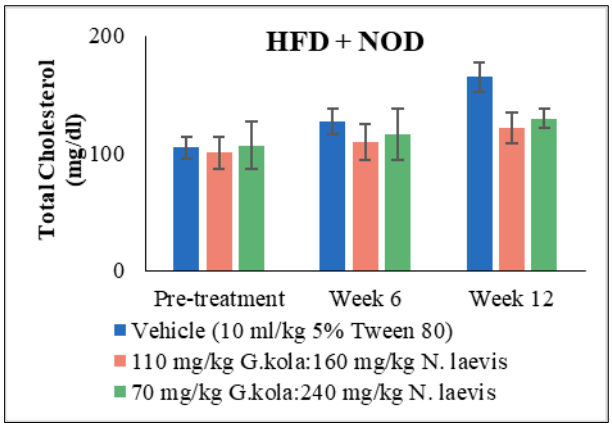

Figure 36 Effect on Total Cholesterol

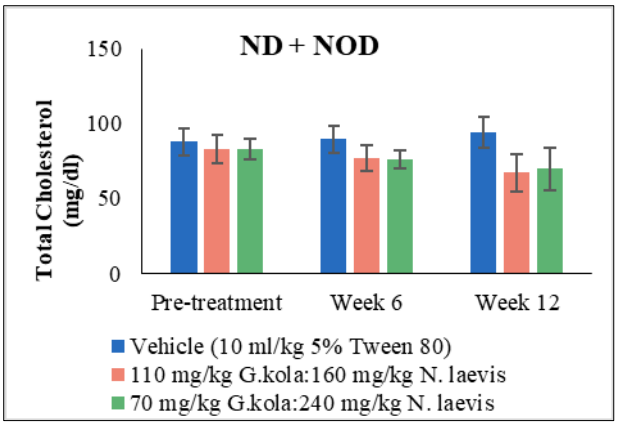

Figure 37 Effect on Total Cholesterol

3.4.2. Triglyceride

High fat diet feeding of diabetic animals produced progressive increase in serum triglyceride concentration that reached significant (P<0.05) level on the 12 th week compared to pre-treatment/basal value (Figures 38,39,40 and 41). Administration of glibenclamide also showed significant (P<0.05) increase in serum triglyceride on the 12th week just like the vehicle control group. However, compared to glibenclamide and vehicle control group the combination doses of *G. kola* and *N. laevis* at the tested combination dose ratios showed significant (P<0.05) reduction in serum triglyceride on the 12th week just like pioglitazone reference antidiabetic standard.

For diabetic animals on normal diet (ND + D), significant (P<0.05) increase in serum triglyceride was also observed on the 12 th week but to a lower magnitude compared to the experimental diabetic group on high fat diet (HFD + D). Glibenclamide administration showed progressive reduction in serum triglyceride concentration with significant (P<0.05) effect occurring from the 6th week compared to vehicle control group. Pioglitazone on the other hand showed significant (P<0.05) reduction compared to both the vehicle control and pre-treatment value from the 6th week. The combination doses of *G. kola* and *N. laevis* showed significantly (P<0.05) lower effect compared to these two reference standards (glibenclamide and pioglitazone) on the 6 th week. However, their effects were similar to the reference control groups on the 12 th week with significant (P<0.05) reduction in serum triglyceride compared to vehicle control and a non-significant (P>0.05) difference compared to glibenclamide. Also the effect produced by 110 mg/kg *G. kola*: 160 mg/kg *N. laevis* combination dose ratio was not significantly (P>0.05) different from that of pioglitazone on the 12 th week.

For non-diabetic animals placed on high fat diet (HFD + NOD), the significant (P<0.05) increase in serum triglyceride produced on the 12 th week was significantly (P<0.05) suppressed by only 110 mg/kg *G. kola*: 160 mg/kg *N. laevis* combination dose ratio. However, both combination doses (110 mg/kg *G. kola*: 160 mg/kg *N. laevis* and 70 mg/kg *G. kola*: 240 mg/kg *N. laevis*) significantly (P<0.05) suppressed serum triglyceride concentration in non-diabetic animals on normal diet.

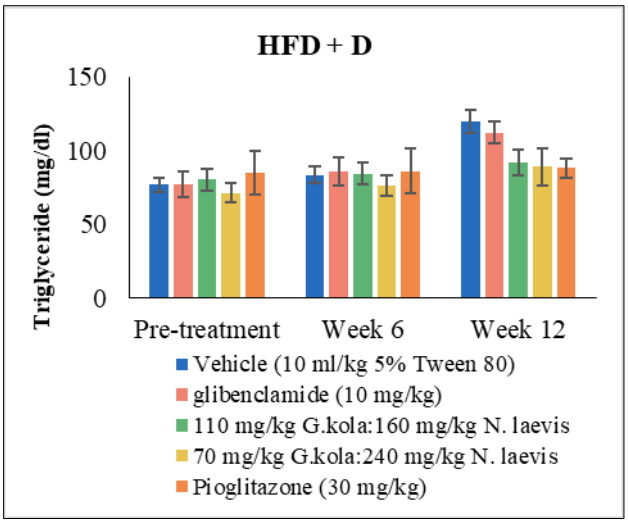

Figure 38 Effect of Treatment on Triglyceride

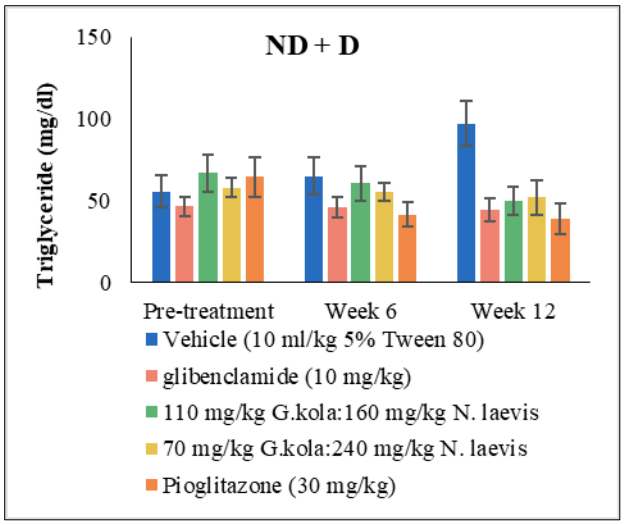

Figure 39 Effect of Treatment on Triglyceride

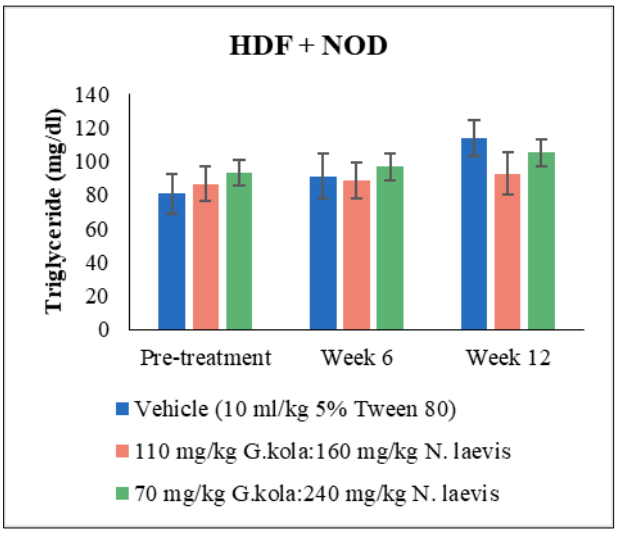

Figure 40 Effect of Treatment on Triglyceride

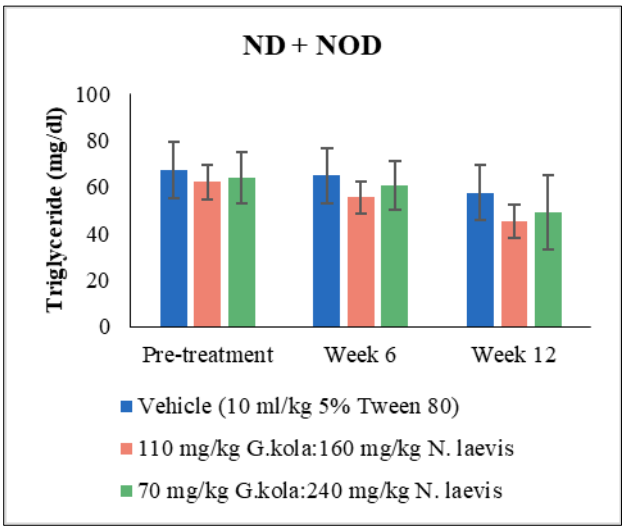

Figure 41 Effect of Treatment on

3.4.3. HDL

Diabetic animals on high fat (HFD + D) diet showed reduction (Figure 42,43,44 and 45) in high-density lipoprotein cholesterol (HDL-C) concentration that reached a significant (P<0.05) level in the 12th week in the vehicle control group. Unlike significant (P<0.05) reduction recorded by glibenclamide treated group, treatment with the combination doses of *G. kola* and *N. laevis* showed significant (P<0.05) increase in serum HDL just like in pioglitazone treated group when compared to both vehicle control and glibenclamide groups. Better effects were produced by the combination doses at both week 6 and 12 compared to other treatment groups. Significantly (P<0.05) higher serum HDL was recorded from treatment with 110 mg/kg *G. kola*: 160 mg/kg *N. laevis* combination dose ratio on the 12 th week compared to pioglitazone.

Diabetic control animals on normal diet (ND + D) also showed significant (P<0.05) reduction in HDL on the 12 th week compared to pre-treatment/basal value. Compared to vehicle control group, treatment with glibenclamide and pioglitazone showed significant (P<0.05) increase in HDL from the 6th week while the increase produced by the combination doses became significant (P<0.05) on the 12 th week. The increase in serum HDL-C produced by the combination doses on the 12 th week was also found to be significant (P<0.05) compared to the pre-treatment value just as with the two reference drugs (pioglitazone and glibenclamide).

Similarly, treatment of non-diabetic animals on high fat diet (HFD + NOD) with the combination doses produced significant (P<0.05) increase in HDL-C concentration on the 12 th week compared to vehicle control group. Also the

increase produced by this treatment in non-diabetic animals on normal diet was significant (P<0.05) compared to both pre-treatment values and vehicle control group.

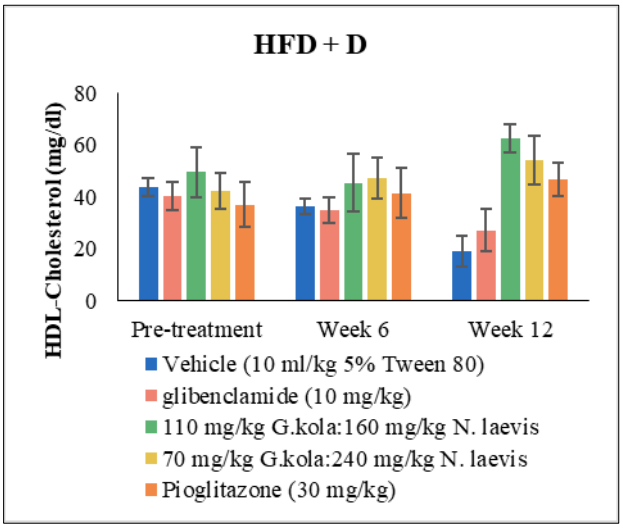

Figure 42 Effect of Treatment on HDL - Cholesterol

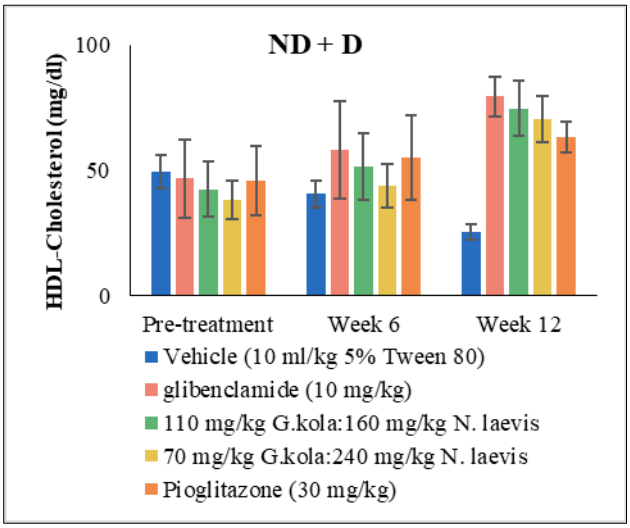

Figure 43 Effect of Treatment on HDL - Cholesterol

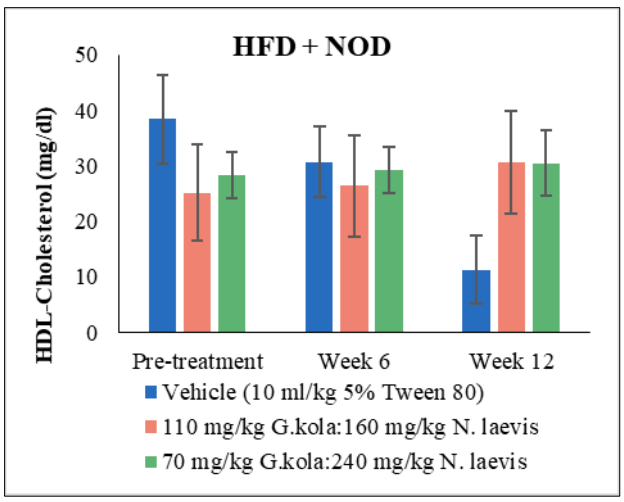

Figure 44 Effect of Treatment on HDL - Cholesterol

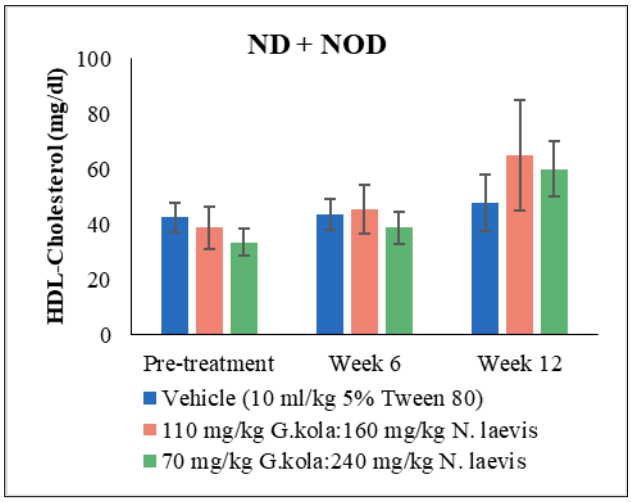

Figure 45 Effect of Treatment on HDL - Cholesterol

3.4.4. LDL

In high fat diet diabetic (HFD + D) experimental animal group, significant (P<0.05) increase in low density lipoprotein cholesterol (LDL-C) in vehicle control group was recorded from the 6th week just as in glibenclamide treated group (Figures 46,47,48 and 49). When compared to pre-treatment/basal values, treatment with the combination doses brought about reduction in these values that were of significant (P<0.05) effect on the 12th week when compared to both vehicle control and glibenclamide treated groups. The effect shown by the combination doses were comparable to that of pioglitazone with no obvious difference occurring between these groups when compared (P>0.05). For the diabetic animals on normal diet (ND + D), significant (P<0.05) increase in LDL-cholesterol concentration was also observed in the vehicle control group from the 6th week. When this control group was compared to the treatment groups, significant (P<0.05) reduction was recorded by the 110 mg/kg *G. kola*: 160 mg/kg *N. laevis* combination dose ratio just as in glibenclamide and pioglitazone reference drug treated groups. Also both combination dose ratios tested showed significant (P<0.05) reduction on the 12th week when compared to vehicle control group. Experimental groups of non-diabetic animal on high fat diet (HFD + NOD) showed significant increase in LDL-C in the untreated vehicle control group from the 6th week. The reductive effect of the combination doses on this experimental group was of significant value on week 12 compared to both pre-treatment value and vehicle control group. For non-diabetic animals on normal diet (ND + NOD), the combination doses produced significant (P<0.05) reduction in LDL-C from week 6 compared to pre-treatment values and on week 12 compared to vehicle control group.

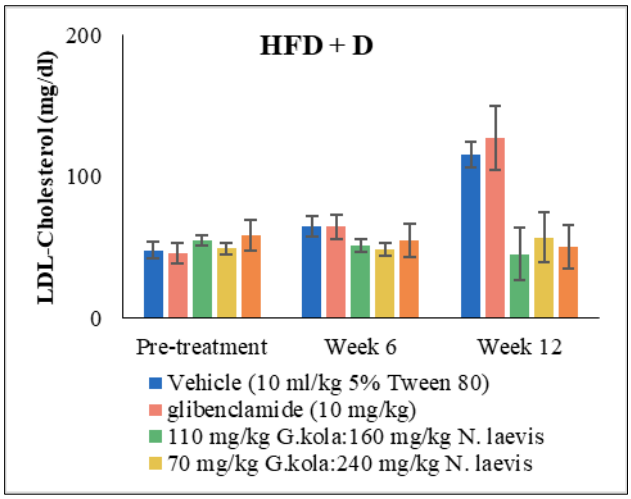

Figure 46 Effect of Treatment on LDL - Cholesterol

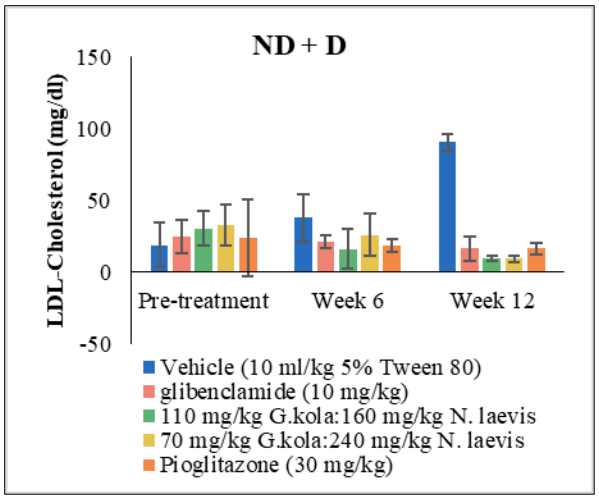

Figure 47 Effect of Treatment on LDL – Cholesterol

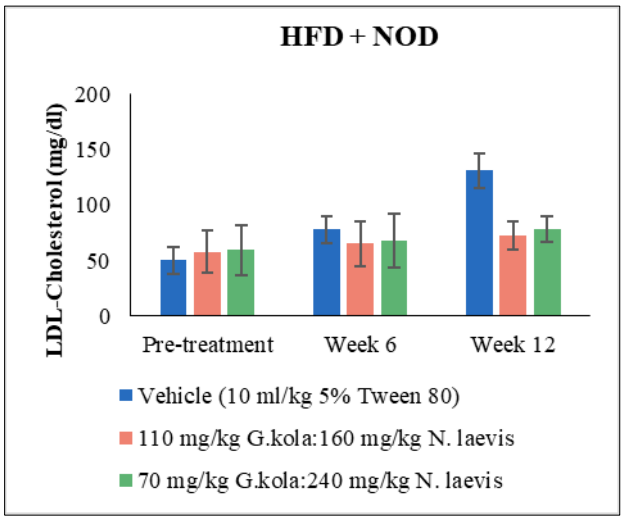

Figure 48 Effect of Treatment on LDL - Cholesterol

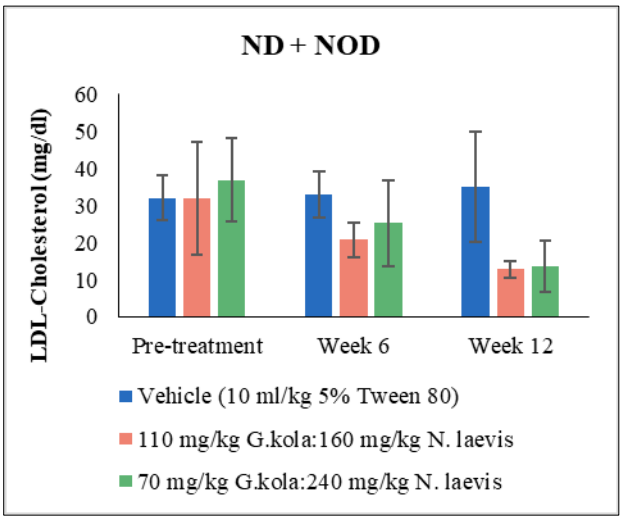

Figure 49 Effect of Treatment on LDL – Cholesterol

3.4.5. VLDL

Vehicle control diabetic animals on high fat diet showed increase in VLDL-cholesterol (Figures 50,51,52 and 53) that become significant (P<0.05) on the 12th week compared to pre-treatment/basal value. Similar increase was also recorded in the glibenclamide treated group. The combination dose treatments just like with the pioglitazone countered the high fat diet diabetes induced increase in VLDL-Cholesterol concentration with significant (P<0.05) effect achieved on the 12th week when compared to vehicle and glibenclamide control groups. Unlike in the HFD diabetic experimental condition, glibenclamide showed opposite effect in normal diet diabetic experimental condition with significant (P<0.05) decrease in VLDL-C recorded from the 6th week just like in pioglitazone treated group compared to vehicle control group. The reduction recorded from treatment with the combination doses of *G. kola* and *N. laevis* was of significant effect on the 12th week compared to vehicle control group. Similarly, only the combination dose ratio of 110 mg/kg *G. kola*: 160 mg/kg *N. laevis* was able to lower VLDL-C concentration in high fat diet non-diabetic animals on the 12th week when compared to vehicle control group while both dose ratios produced significant (P<0.05) reduction in normal diet non-diabetic (ND+NOD) experimental group on week 12 when compared to pre-treatment values.

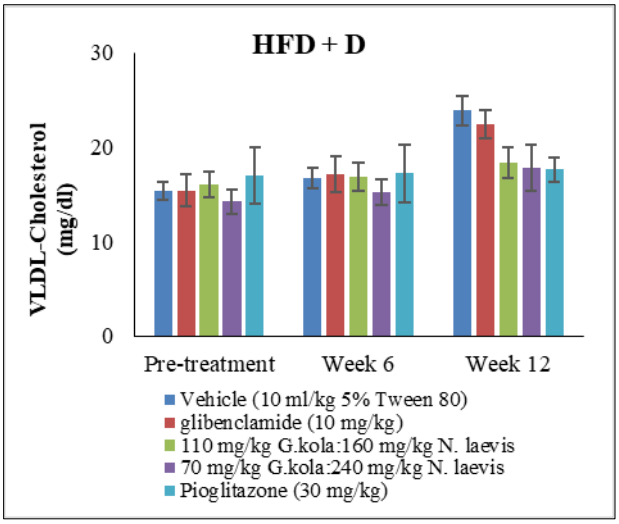

Figure 50 Effect of Treatment on VLDL - Cholesterol

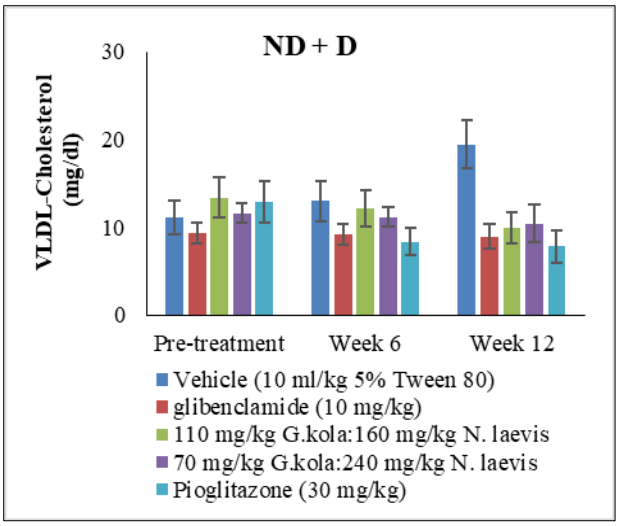

Figure 51 Effect of Treatment on VLDL – Cholesterol

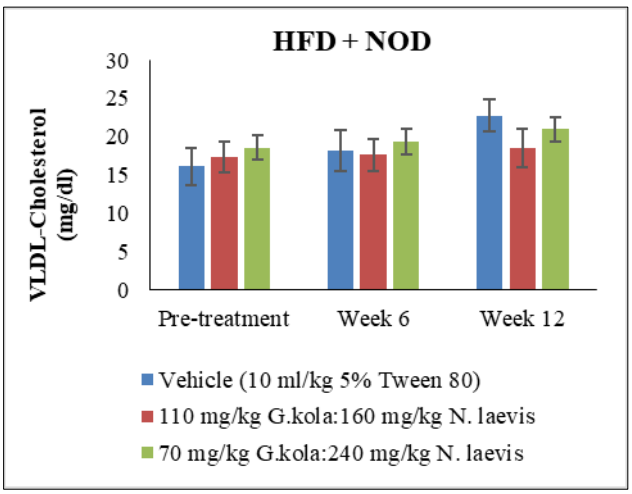

Figure 52 Effect of Treatment on VLDL - Cholesterol

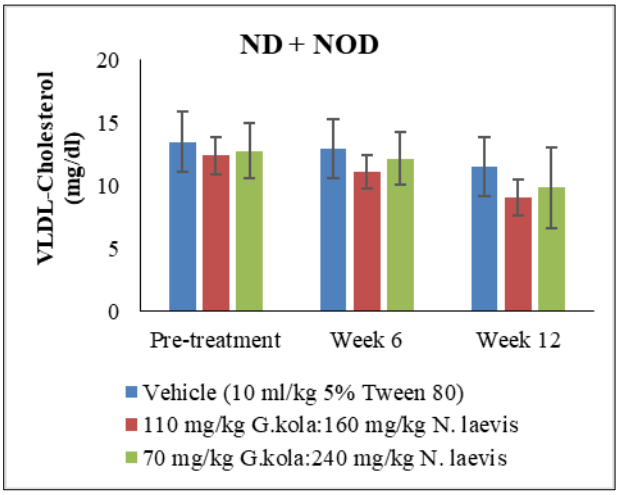

Figure 53 Effect of Treatment on VLDL – Cholesterol

4. **Discussion**

According to the Globally Harmonized System (GHS) toxicity classification, substances with oral LD_{50} in the range 2000–5000 mg/kg BW have relatively low toxicity (UN GHS, 2019). The LD_{50}, being greater than 5000 mg/kg b.w., is thought to be safe. In the present study the administration of both extracts up till 5000 mg/kg did not produce mortality, though at higher doses of 4000 and 5000 mg/kg, the physical activities of the animals were reduced after the administration. Iniaghe *et al* (2015)(23) reported that the oral LD_{50} was estimated to be more than 5000 mg/kg showing that the extract is relatively safe for use. According to Okonkwo *et al* (2022)(13), Oral administration of the crude methanol G. kola extract at a dose between 10 mg/kg and 5000 mg/kg produced no toxic signs or mortality. The low extract dose levels (150 mg/kg, 300 mg/kg) of G. kola extract exhibited no significant effect on the histomorphology and gross anatomy of the vital organs. However, at a high dose (600 mg/kg, the extract exhibited a significant decrease in the weight of the liver and kidney in comparison with the control (24). The median lethal dose was therefore considered as ≥ 5000 mg/kg. This explains the safety in the chronic use of G. kola in a chronic disease like diabetes. The report of Owolabi et al. (2011) on the Effect of Ethanol Leaf Extract of *N. laevis* on Blood Glucose Levels of Diabetic Rats revealed that the median lethal dose (LD_{50}) was 6 g/kg for the intraperitoneal route, and indeterminate for the oral route and is in line with the report of the present work of LD_{50} of up to 5000 mg/kg of the *N. laevis* leaf extract. This too explains the high safety profile observed in the chronic use of *N. laevis* for a chronic disease like diabetes. Effective doses on Day 30 of treatment with *G. kola* was 262 mg/kg

while that of *N. laevis* was 404 mg/kg which shows that about 5.24 % of LD_{50} of *G. kola* and 8.08 of LD_{50} of *N. laevis* were used in the study. The differences in the two extracts could be explained by their differences in the phenolic content.

Induction of diabetes with Streptozotocin decreases Nicotinamide-adenine dinucleotide (NAD) in pancreas islet beta cells and causes histopathological effects in beta cells which probably intermediates induction of diabetes (25). In IDDM, insulin-producing beta cells of the pancreatic islets (PIs) are progressively destroyed, insulin production is reduced, and the plasma insulin level becomes extremely low (26). Earlier studies show that nicotinamide protects against the diabetogenic effect of STZ. This compound combination produces a model of insulin-deficient, but not insulin-resistant, T2D. It is characterized by stable, moderate hyperglycemia associated with an approximately 60 % loss of β-cell function In this case, the animals are exposed to a high-fat diet to produce insulin resistance, followed by administration of a moderate dose of STZ to reduce β-cell capacity. The result is hyperglycemia, associated with hyperinsulinemia and insulin resistance. (27). Chemical agents, such as streptozotocin (STZ) and alloxan, that can selectively damage the insulin-producing β-cells in the pancreas resulting in hyperglycaemia, are important tools for developing animal models of diabetic complications. To be relevant to humans, animal models must replicate the phenotype and mimic the developmental process of the disease. Thus the establishment of the T2DM model was achieved by feeding HFD to produce insulin resistance, followed by low dose of STZ injection to cause mild β-cell dysfunction (28)

In accordance with the reports above, STZ-NAD induced type-2 diabetes was achieved in the animal with FBS above 160 mg. There is therefore reduction in insulin production by the beta-pancreatic cells and the plasma insulin level. Reactive oxygen (ROS) and reactive nitrogen species (RNS) are highly reactive oxidized molecules, which are generated constantly by normal cellular conditions, for instance the activity of the mitochondrial respiratory chain and inflammation, which could lead to damage in other biological molecules, like proteins and DNA (29). Hyperglycemia and dyslipidemia can induce inflammatory responses, in the meantime producing free radicals, which lead to type 2 diabetes and cardiovascular complications. While genetic models are expensive and do not accurately represent human T2DM, the most used animal model for T2DM involves feeding rodents an HFD and injecting them with a low dose of streptozotocin (STZ; approximately 35 mg/k) (28). Researchers have shown that combining an HFD with a low-dose STZ treatment can effectively create a rat model that mimics the natural progression and metabolic features of typical T2DM in humans. This model is cost-effective, easy to develop, and well suited for studying the underlying mechanisms of T2DM and evaluating potential therapeutic compounds for the treatment of T2DM and its associated complications (30). T2DM and its associated complications arise from insulin resistance and β-cell dysfunction, making it a complex condition. The disease progresses through two stages: compensation and decompensation. During the compensation stage, the body compensates for insulin resistance by increasing insulin secretion to maintain euglycemia. However, as the disease advances, the body becomes unable to reduce enough insulin to meet the demand, leading to hyperglycemia, frank diabetes, and ketosis (31). T2DM is recognized as a chronic inflammatory disease, with an unhealthy diet being one of its causative factors. Hyperglycemia is its major symptom and is also considered to be the initiator of the inflammatory micro environment and subsequent complications (32).

In the STZ-NAD induced diabetic animals fed with HFD, the diabetic condition of the animals therefore mimics ROS stress-induced diabetes with deficient insulin production and hyperglycemic-induced insulin resistant diabetes. The observed reduction in FBS, lipid profile, increase in insulin secretion, HOMA-IR values and OGTT, in the animals treated with the extracts or the combination is based on their high phenolic contents. The reported relationship between phenolic compounds in antioxidant and anti-inflammatory effects may explain their activities in diabetes and diabetic complications. These effects may be based on their protective effects on the pancreatic beta cells and improvement on cellular signaling that modulates insulin resistance. Phenolic phytochemicals have also been found to have potential in the management of oxidative stress-linked chronic diseases, like diabetes, cancer and cardiovascular disease (33). Plant polyphenols can improve the endogenous antioxidative system, effectively prevent oxidative damage and improve oxidant-antioxidant balance. The research on green tea shows that it contains six primary catechins as the polyphenolic compounds that are the most common in this field; and these bioactive components reduced lipid peroxidation and increased plasma total antioxidant capacity. Stress-sensitive signaling pathways, preoxidant enzymes and the induction of antioxidant enzymes, including superoxide dismutase, catalase and glutathione peroxidase, were also decreased (34). Phenolic compounds inhibit the expression of the inducible nitric oxide synthase gene and inhibit the activity of transcription factor NF-kB. Therefore, nitric oxide (NO) products can be prevented, and the protective effect of insulin-producing rat pancreatic cell line (RINm5F) and pancreas on insulin secretion and secretion and islet beta cell activity can be maintained. In addition, natural plants, like cinnamon extract, can activate glycogen synthase and insulin receptor kinase, increase in vivo glucose uptake, inhibit glycogen synthase kinase-3 beta, inhibit the dephosphorylation of the insulin receptor and improve insulin sensitivity. (35).

The active phytochemical constituents of individual plants are inadequate to attain the desirable therapeutic effects. When polyherbal and herbo-mineral formulations combining the multiple herbs in a meticulous ratio, it will give an

enhanced therapeutic effect and decrease the toxicity. The active constituents used from individual plant are inadequate to provide attractive pharmacological action. There are evidences that crude plant extracts often have greater potency rather than isolated constituents (36). Combination of herbs have proven more efficacious in multi-targeted diseases, as compared to individual herbs owing to the "effect enhancing and side-effect neutralizing properties of herbs, which forms the basis of polyherbal therapies (37) and is in line with the observed more FBS reducing capacity of the combination ratios than the individual extracts even in additivity combination. This may be as a result of some interactions between the varied phytoconstituents of the extracts. A synergistic effect observed in the combination of the two extracts produced a lower IC_{50} of 2.2 which was observed to be more potent than the individual extracts that produced the IC_{50} of 2.757 and 2.845 for *G.kola* and *N.laevis* extracts respectively. Polyherbal formulation offers a better therapeutic effect compared with a single multi-component drug (36), through multiple bioactive compounds following multiple interactions both at cellular and molecular levels, hence the observed synergism in the course of this study.

5. Conclusion

This study has revealed the potentials of *G.kola* and *N. laevis* combination in combating the global health challenge posed by type 2 diabetes and its associated complications. The combination has shown a very significant activity in regulation of both sugar and fat homeostasis through pancreatic cell regenerative and insulin sensitivity properties. These activities are mediated possibly through anti-oxidative effect, reduced gastric emptying rate with associated improvement in oral fat and glucose tolerance. This combination has potentials in reducing both body weight and food consumption and therefore serve as effective formulation against obesity and hyperlipidemia, conditions that predispose a very large global population to intractable chronic diseases including cancers, kidney failure and other organopathies. This is the first study on the effective combination dose ratio of *G. kola* and *N. laevis* with insights into the possible bioactive constituents. The synergistic effect that was observed in the combination of the two extracts gives hope for a novel drug that will ultimately emancipate humanity from the woes of diabetes and its associated complications.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest as regard this research

Statement of ethical approval

Ethical approval was taken from the institutional ethics committee prior to the start of study.

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