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Original Research Article

Co-administration Effects of *Newbouldia laevis* (P. Beauv.) Leaves and *Garcinia kola* (Heckel) Seed Extracts on Animal Models of Hepatotoxicity and Diabetes

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ABSTRACT

Newbouldia laevis (P. Beauv.) leaves and *Garcinia kola* (Heckel) seeds have been demonstrated to have therapeutic applications in the treatment of diabetes and hepatic disorders. The study investigated the effects of co-administration of both plants in the treatment of diabetes and hepatopathy. The effects of the methanol extracts on alloxan-induced diabetes were determined singly and in combination at 200 and 400 mg/kg for 10 days. Similarly, the extracts and their combinations at the same doses were tested on CCl_4 induced liver damage. On day 5, co-administration at 200 and 400 mg/kg produced a significant ($P < 0.05$) reduction in blood glucose compared with the post-induction values. Synergistic interaction between the co-administration at both doses was recorded on day 5 with a combination index of 0.63 at 400 mg/kg and 0.64 at 200 mg/kg. The co-administration produced near additive effect in the reduction of diabetes-induced elevation of ALT enzyme concentration with combination index of 1.43 at 400 mg/kg and 1.29 at 200 mg/kg. For the CCl_4 induced liver oxidative damage, combination of both extracts produced a synergistic reduction in serum ALT concentration at both 200 and 400 mg/kg (Combination index of 0.65 and 0.89 respectively). At lower dose (200 mg/kg) combination therapy, there was synergistic reduction of serum AST (combination index of 0.83) and additive reduction at 400 mg/kg (combination index of 1.14). The combination of both extracts produced positive interaction both in the reduction of blood glucose and protection against oxidative liver damage with higher synergism recorded at lower concentration.

Keywords: Diabetes, Hepatotoxicity, Drug interaction, *Newbouldia laevis; Garcinia kola*.

Introduction

Diabetes mellitus is a chronic metabolic disease that is characterized by hyperglycemia, inadequate production of insulin, or inadequate sensitivity.[1] The disease leads to reduced quality of life and increases risk factors for mortality and morbidity.[2] International Diabetes Federation reported that the estimated number of adults living with diabetes reached 463 million in 2019 with 10% of global expenditure spent on diabetes.[3]

Oxidative stress has been implicated in the pathogenesis of diabetes and its associated complications. Elevations of pro-oxidants and oxidative tissue damage markers have been documented to be increased in serum, plasma, white blood cells, and pancreas biopsies of patients with type II diabetes.[4]

Medicinal plants have been used since ancient times for the treatment and management of diabetic mellitus (DM) in traditional medicine systems of many cultures throughout the world.[5]

Despite tremendous advances in chemotherapy, no reliable drugs have been developed that protect the liver from damage and/or help in the regeneration of hepatic cells. Modern medicines therefore, have little to offer for the alleviation of hepatic diseases and it is chiefly the plant based preparations which are employed for the treatment of liver disorders.[6,7]

Newbouldia laevis (P.Beauv) is an angiosperm that belongs to the Bignoniaceae family. Its common names are fertility tree and African border tree.[8.] In Nigeria, it is known with different names among different communities: 'Aduruku', 'Ogirisi', 'Ikhimi', and 'Akoko' by the Hausa, Igbo, Bini and Yoruba respectively.[9] *Garcinia kola* (Heckel) Guttiferae is a dicotyledonous plant found in most forest and grows as a medium sized tree up to about 12m high.[10] It is used in traditional medicine for the treatment of ailments such as liver disorders, diarrhea, laryngitis, bronchitis and gonorrhea.[11] Although, *G. kola* has been reported to produce reductions in hyperglycermia and hypercholesterolemic in diabetic animals and to protect against oxidative stress induced by toxins in experimental animal models,[12] its efficacy in combination with *Newbouldia laevis* in diabetes and chemical induced hepatopathy remains unexplored. The study investigated the effects of co-administration of *N. laevis* and *G. kola* in both diabetes and hepatopathy.

Materials and Methods

Plants

The leaves of *Newbouldia laevis* and seed of *Garcinia kola* were obtained from Agulu Anambra State Nigeria in August, 2018. The authentication of the plants was done by a taxonomist Mr. Nwafor Felix of the Department of Pharmacognosy and Environmental

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Medicine, University of Nigeria, Nsukka. The Voucher specimens were deposited at the herbarium of the Faculty of Pharmaceutical Science, Nnamdi Azikiwe University, Agulu Campus, under the herbarium number PCG/474/A/035 for *N. laevis* and PCG/474/A/046 for *G. kola.* The plant materials were air dried and pulverized with mechanical grinder (Gx160 Delmar 5.5HP, Honda Motor CO., LTD, Japan) into coarse powder.

Animals
Adult Wistar rats (150 – 180 g) and Swiss mice (20 – 25 g) used for the study were obtained from the Animal House of the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology. The animals were fed with pelletized feed (Vital Feeds, Nigeria) and had access to water *ad libitum.* Housing of the animals was done in standard experimental conditions in the Animal House of the Department of Pharmacology. The animals were allowed to acclimatize for 3 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 as described in the animal experimental protocol approved by the Institution Animal Care and Use Committee (ESUT/FPS/PHA/2018/009).

Extraction
The pulverized leaves of *N. laevis* (1 kg) and seeds of *G. kola* (1 kg) were separately macerated in 5 L of 70% methanol for 72 h with intermittent shaking as described by Zhang *et al*.[13] The resulting solution was filtered, and the filtrate was concentrated *in vacuo* at 40°C to obtain the methanol extract.

Phytochemical and total phenolic content determination
The qualitative phytochemical analysis of the extracts was carried out using standard methods. [14] While Folin ciocalteu's method was used to determine the total phenolic content. [15]

Acute toxicity study
The acute toxicity test was carried out using Lorke's method, [16] with slight modification. The test was performed in two phases. In the first phase, 3 groups of 3 mice each were given by oral route 10, 100 and 1000 mg/kg respectively of the extract. The animals were observed for 24 hours post administration for signs of toxicity and mortality. In the second phase, 4 mice were administered orally 2000, 3000, 4000 and 5000 mg/kg of the extract respectively. The animals were again observed for signs of toxicity and mortality for 24 hours. The same procedure was applied for each of the extracts.

Induction of diabetes
Diabetes was induced in 60 Wistar rats by a single intraperitoneal injection of 150 mg/kg alloxan monohydrate according to the previously described method.[17] Basal fasting blood glucose level was determined pre-induction and 72 h post-induction through tail vein puncture using One Touch Glucometer (Lifeshield, Johnson & Johnson, California). Rats with fasting blood glucose level of 160 mg/dl and above were taken to be diabetic and used for the anti-diabetic study.

Acute antidiabetic study
Diabetic rats were randomly divided into 8 groups of 5 animals per group. Groups 1and 2 received 200 and 400 mg/kg of *N. laevis* extract, 3 and 4 received 200 and 400 mg/kg *G. kola* extract, 5 and 6 received equal ratio combination of 200 and 400 mg/kg of *N. laevis* and *G. kola* respectively. Group 7 received 5 mg/kg glibenclamide, while group 8 received 5 ml/kg of 5% Tween 20. Treatments were given by oral route daily for 10 days. Blood samples were collected from the tail vein after an overnight fast at intervals of 0, 5 and 10 days for the determination of glucose concentration using One Touch Glucometer. On the 10th day, blood samples were also obtained from the retro-orbital plexus for the determination of serum liver enzymes levels.

Determination of liver function enzymes levels
Blood samples were allowed to clot for 30 minutes and centrifuged at 3000 rpm for 10 minutes. The serum (supernatant) was used for the assays. Serum enzyme activities of Alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase were determined using Randox kits as per manufacturer's guidelines.

CCl_4 induced oxidative stress
The method reported by Obioha et al.,[18] was used for this study. Eight groups of 5 animals per group were used. Groups 1and 2 received 200 and 400 mg/kg of *N. laevis* extract, 3 and 4 received 200 and 400 mg/kg G. kola extract, 5 and 6 received equal ratio combination of 200 and 400 mg/kg of *N. laevis* and *G. kola* respectively. Group 7 received 50 mg/kg silymarin, while group 8 received 5 ml/kg of 5% Tween 20. Treatments lasted for 10 days. Two hours after the 10th day treatment, the animals were administered a single intraperitoneal dose of CCl_4 (2 ml/kg), in a 1:1 ratio with o olive oil. Blood samples were collected from the animals through the retro-orbital plexus 8 h after CCl_4 administration for the determination of liver oxidative damage. Serum liver function marker enzymes – alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were used to monitor the extent of liver oxidative stress.[19] These tests were performed with serum before treatment, post-treatment and post-induction of CCl_4 induced oxidative damage.

Assessment of synergy in the combination therapy
Fixed ratio combination synergy between *N. laevis* and *G. kola* extracts was determined using an effect-based strategy.[20] Percentage effect value of the extracts at different concentrations separately and when combined were used for the calculation of their combination index using the formula.

$$\frac{A}{AB} + \frac{B}{AB} = X$$

Where A = Effect of *N. laevis*, B = Effect of *G. kola*, AB = Effect of the equal ratio combinations of *N. laevis* and *G. kola*. When X = 1 (additive interaction), X > 1 (antagonistic interaction) and X < 1 (synergistic interaction).

Statistical analysis
Experimental data obtained were presented as mean ± standard error of mean (SEM). Analysis of variance (ANOVA) using SPSS 18.0 was used to analyze differences between means. P values < 0.05 were taken to be statistically significant. Multiple post-hoc comparison was done using Turkey's test. Graphical plots were done using Microsoft Excel 2010.

Results and Discussion

Extraction, yield and phytochemical analysis
Methanol extract of *N. laevis* produced higher yield compared with *G. kola* (Table 1). Both extracts showed an abundance of saponins, glycosides, steroids and terpenoids with the absence of alkaloids. High total phenolic content was recorded from both extracts with *G. kola* having more than twice the phenolic content of *N. laevis*.
One-drug, one-target development model has achieved great success, however, its limitations have become evident especially in complex disease conditions such as diabetes mellitus with its associated complications.[21] These complex disease conditions result from the interacting outcomes of multiple pathways thus requiring multiple approaches for treatment. One of the proposed effective approaches is exploring the potentials of drug combination therapy.
Phenolic compounds like flavonoids and tannins have extensive application in the treatment of hepatotoxicity and diabetes. Some of these applications include decreases in lipid peroxidation, increases in antioxidant enzymes (like SOD, GPX, and CAT) activities, inhibition of insulin-dependent activation of PI3K, and reduction in intestinal

glucose absorption by inhibiting GLUT2.[22,23] Blockage of tyrosine kinase is another mechanism by which flavonoids is reported to have effects against diabetes. Eid et al.[24] reported that quercetin stimulates GLUT4 translocation and expression in skeletal muscle. The presence of these phytocompounds may be attributed to the observed antidiabetic and hepatoprotective effects of *G. kola* and *N. laevis.*

Acute toxic effect of the extracts

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 (movement and feeding) of the animals were reduced after the administration but normalized 2 h post administration. Lack of obvious signs of toxicity suggests that the plant extracts may be safe for the treatment of diabetes and its associated hepatopathy.

Effects of the extracts on blood glucose and diabetes induced oxidative stress

Persistent hyperglycemia and increased oxidative stress are major players in the development of secondary diabetic complications such as hepatic injury.[25] Alloxan monohydrate induced significant ($P < 0.05$) increase in blood glucose across the groups however, treatment with the extracts produced dose dependent reduction in blood glucose at days 5 and 10 (Figure 1). Progressive reduction in blood glucose with time was also observed with various treatments. On day 5, only the combination therapies at 200 and 400 mg/kg produced significant ($P<0.05$) reduction in blood glucose compared with the post-induction values (Figure 2). The combination therapies also produced a significant reduction in blood glucose on day 10. Significant ($P<0.05$) difference at day 5 was also observed between *N. laevis* at 400 mg/kg compared with the combination therapy at the same dose an indication of the potentiating effect of *G.kola* on the hypoglycermic effect of *N. laevis*. Combination therapy at 400 mg/kg was the only therapy with significant ($P<0.05$) reduction in blood glucose compared with the vehicle control group on both days 5 and 10. Synergistic interaction between the combination therapy at both doses was recorded on day 5 with a combination index of 0.63 at 400 mg/kg and 0.64 at 200 mg/kg. Daily treatment with the combination therapy on the 10th day produced approximately additive effect with a combination index of 1.07 at 400 mg/kg and 1.18 at 200 mg/kg.

Similar to alloxan induced hyperglycemia, there was significant ($P < 0.05$) induction of hepatic oxidative damage as indicated by the elevation of serum liver function enzymes. *Garcina kola* at 400 mg/kg and the combinations of both extracts at 200 and 400 mg/kg produced a significant reduction in serum ALT concentration when compared to post-induction and vehicle control values (figure 3). Significant reduction in AST and ALP serum concentration was also recorded for both treatment groups when compared to post-induction and vehicle control groups (figures 4 -8). The combination therapy produced a near additive effect in the reduction of ALT enzyme concentration with a combination index of 1.43 at 400 mg/kg and 1.29 at 200 mg/kg. This additive interaction was only produced at a lower dose (200 mg/kg) for the AST and ALP serum enzyme reduction with combination index of 1.40 and 1.36 respectively compared to corresponding 1.74

Several animal models using Wistar rats have been used to induce diabetes mellitus. The cytotoxic action of alloxan is mediated by reactive oxygen species.[26] Alloxan and the products of its reduction, dialuric acid**;** establish a redox cycle with the formation of super oxide radicals. These radicals undergo dismutation to hydrogen peroxide.[27] Then highly reactive hydroxyl radicals are formed by Fenton reaction.[27] The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells, hence precipitating experimental diabetes mellitus mediated by oxidative stress.[27] This model was chosen based on the documented implication of oxidative stress in the development of diabetes mellitus and its associated complications like hepatopathy.[28] The observed effect of *G. kola* on diabetes is in accord with several reports on the hypoglycermic effect of *G. kola.*[29,30] Similarly, many studies have also demonstrated the antidiabetic and hepatoprotective effects of *N. laevis*.[31] Some of the reported mechanisms include a decrease in lipid peroxidation, an increase in antioxidant enzymes, increase in insulin sensitivity, inhibition of α-amylase enzyme activity, inhibition of gastric emptying, increase in oral glucose and fat tolerance.[32] *N. laevis* has also been documented to attenuate dRib induced cell damage in pancreatic β-cells via oxidative stress-related signaling,[33] preservation of the cellular architecture of vital tissue towards normal in STZ-induced diabetic rats,[33] enhanced GLUT4 translocation upon, and increased liver and muscle glycogen contents.[33]

Effects of the extracts on CCl_4 induced oxidative stress

CCl_4 caused marked increase in serum concentration of liver enzymes. Dose-dependent reductions in these enzymes concentrations were observed in the three liver enzymes. Treatment with *Garcina kola* at both doses, produced significant ($P < 0.05$) reduction in serum ALT, AST, and ALP concentration similar to 50 mg/kg silymarin (Figures 9-11). *Newbouldia laevis* at 400 mg/kg produced significant ($P < 0.05$) reduction in serum AST concentration and at both doses in ALP serum concentration. A combination of both extracts produced positive pharmacodynamics interaction with synergistic reduction in serum ALT concentration produced at both 200 and 400 mg/kg (Combination index of 0.65 and 0.89 respectively). At lower dose (200 mg/kg) combination therapy, there was synergistic reduction of serum AST (combination index of 0.83) and additive reduction at 400 mg/kg (combination index of 1.14). The additive effect was also recorded on the reduction of serum ALP concentration at both doses with a combination index of 1.39 at 200 mg/kg and 1.43 at 400 mg/kg. The synergistic effects of the combination of *N. laevis* and *G. kola* could be as a result of contributive mechanisms of the phytocompounds present in both extracts acting especially on different targets. From the findings of this study, *G. kola* seems to be mediating a potentiation of antidiabetic and hepatoprotective effects of *N. leavis*. Higher activities recorded by *G. kola* when compared with *N. laevis* could be attributed to its higher total phenolic content. The interesting outcome of the combined effect was that lower doses produced better synergistic conclusions than higher doses which is expected to add to the safety of combining both extracts for chronic management of diabetes mellitus.

Table 1: Extraction, phytochemical analysis

Phytocompounds	***G. kola***	***N. laevis***
Saponins	+	+
Cardiac glycosides	+	+
Tannins	+	+
Flavonoids	+	+
Alkaloids	-	-
Steroids	+	+
Terpenoids	+	+
Total Phenolic content (mg/g GAE)	855.82	373.91
Yield (%)	9.17%	14.26%

+ = present; - = absent

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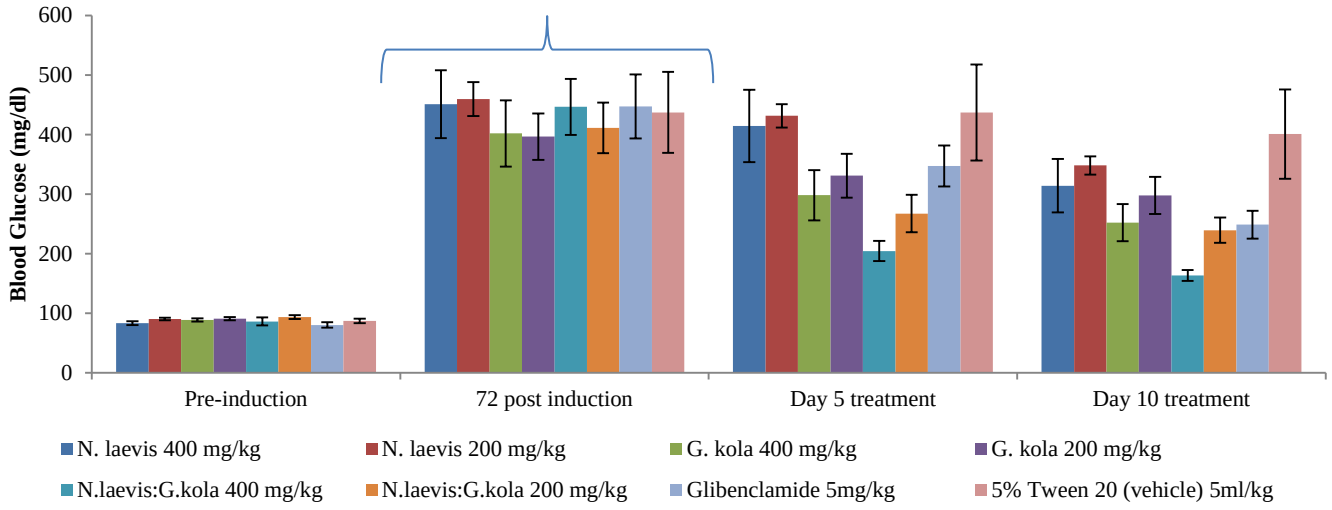

Figure 1: Effect of the extracts of *N. laevis* and *G. kola* on blood glucose of alloxan induced diabetic rats
*P < 0.05 Compared with post induction; # compared with combination therapy at same dose; compared with vehicle control, δ compared with pre-induction

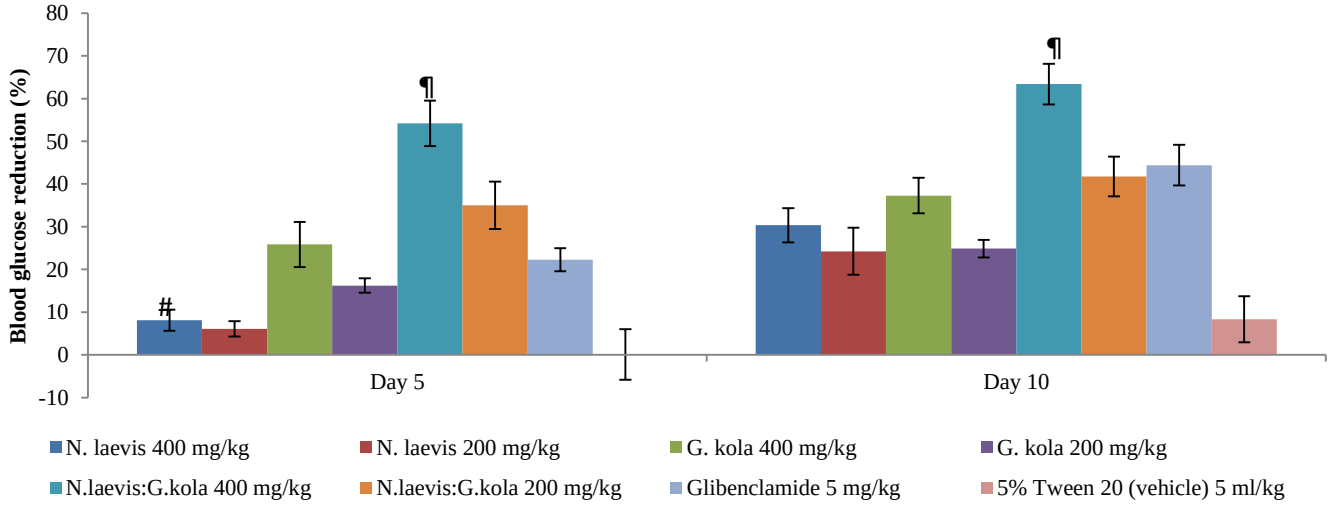

Figure 2: Percentage blood glucose reductive effect of the extracts of *N. laevis* and *G. kola* on alloxan induced diabetic rats
compared with combination therapy at same dose; compared with vehicle control

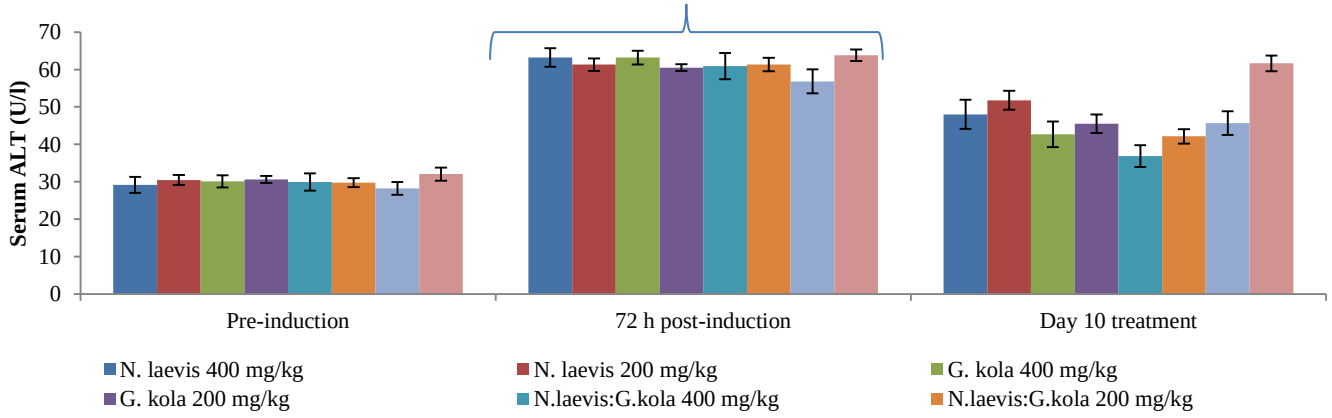

Figure 3: Effect of the extracts of *N. laevis* and *G. kola* on serum ALT concentration of alloxan induced diabetic rats
*P < 0.05 compared with post induction; #P < 0.05 compared with 5% Tween 20 (vehicle), δ compared with pre-induction

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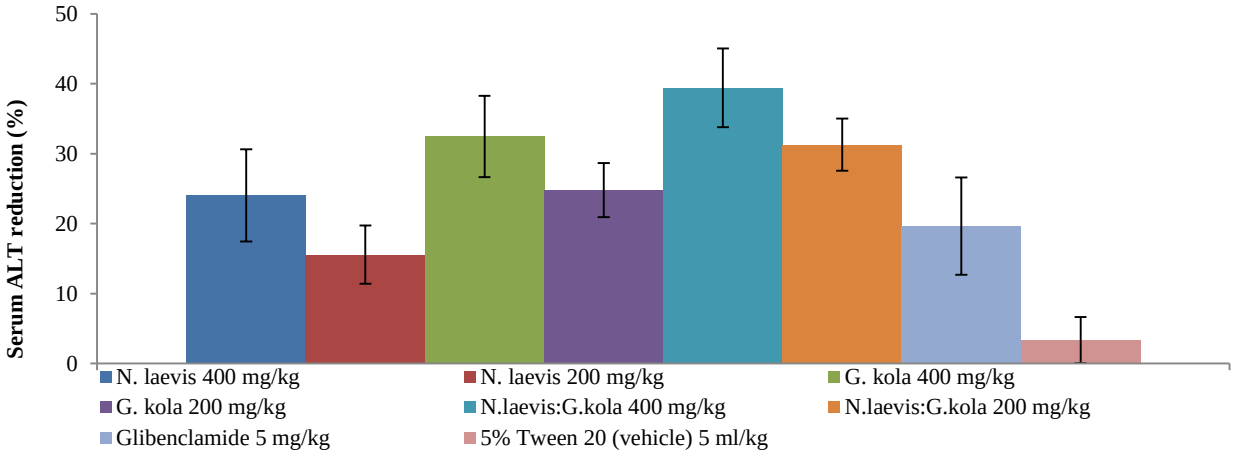

Figure 4: Percentage reductive effect of the extracts of *N. laevis*and*G. kola* on serum ALT concentration of alloxan induced diabetic rats. *P < 0.05 compared with 5% Tween 20 (control)

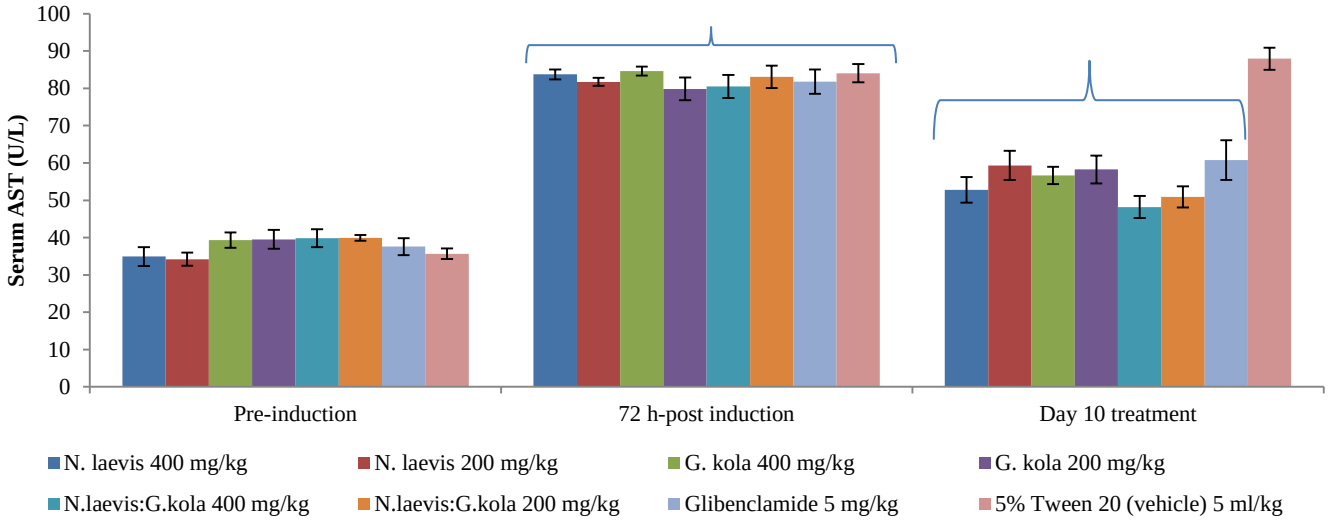

Figure 5: Effect of the extracts of *N. laevis* and *G. kola* on serum AST concentration of alloxan induced diabetic rats *P < 0.05 compared with post induction; #P < 0.05 compared with 5% Tween 20 (vehicle)

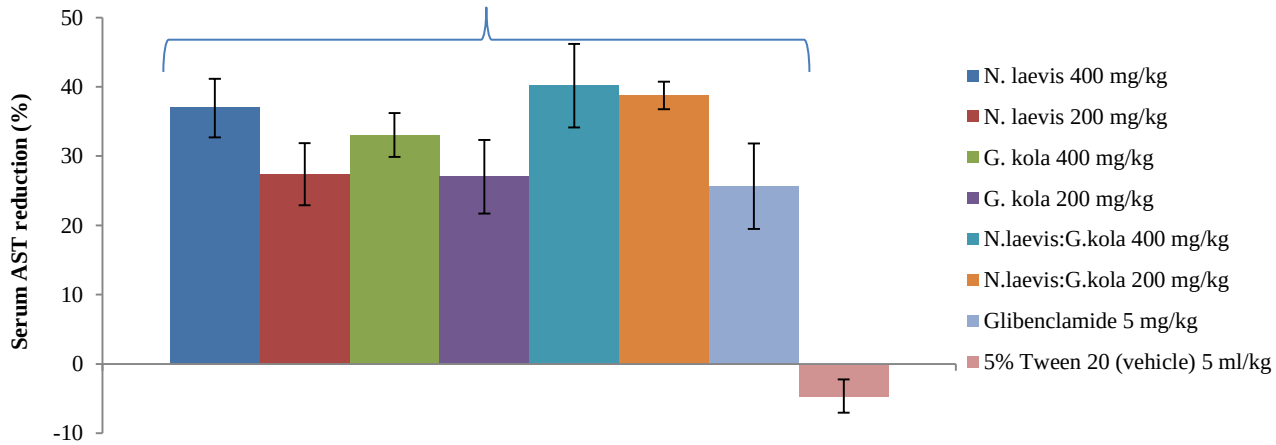

Figure 6: Percentage reductive effect of the extracts of *N. laevis* and *G. kola* on serum AST concentration of alloxan induced diabetic rats. *P < 0.05 compared with 5% Tween 20 (vehicle)

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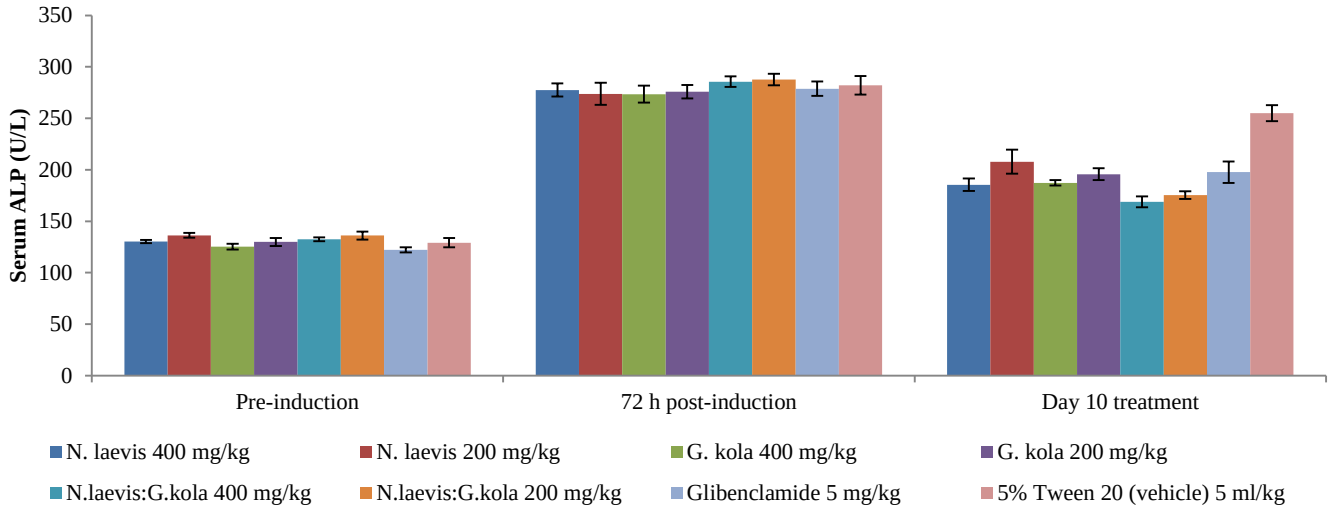

Figure 7: Effect of the extracts of *N. laevis* and *G. kola* on serum ALP concentration of alloxan induced diabetic rats
*P<0.05 compared with post induction; #P<0.05 compared with 5% Tween 20 (vehicle)

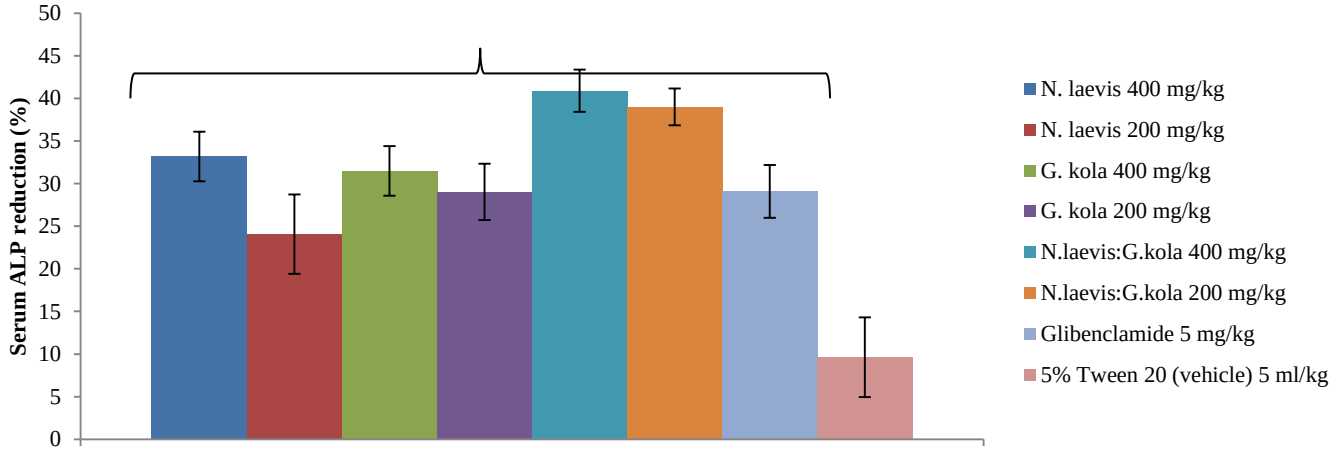

Figure 8: Percentage reductive effect of the extracts of *N. laevis* and *G. kola* on serum ALP concentration of alloxan induced diabetic rats. *P < 0.05 compared with 5% Tween 20 (vehicle)

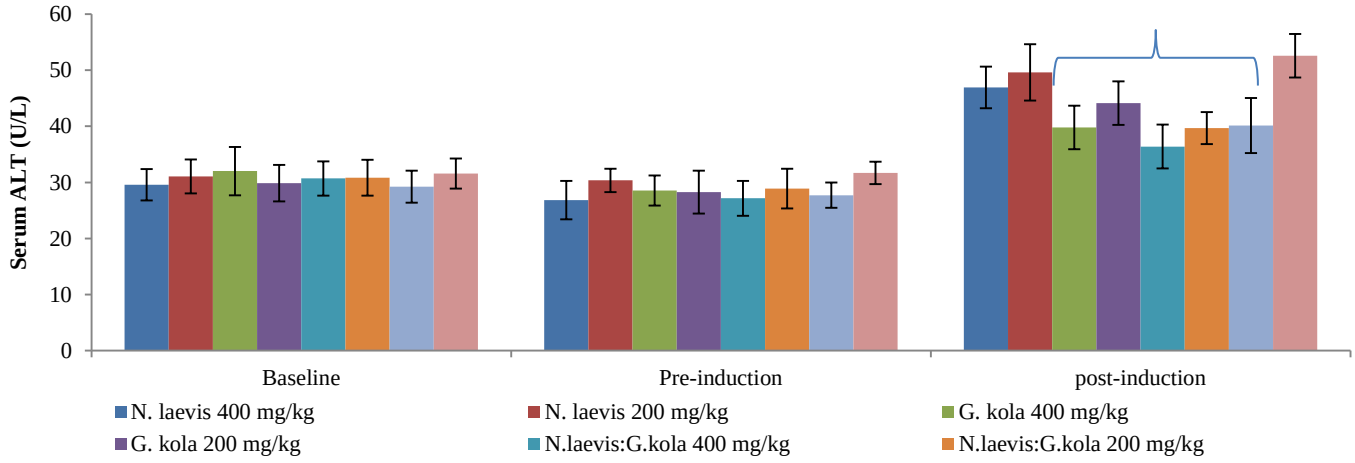

Figure 9: Effect of the extracts of *N. laevis* and *G. kola* on serum ALT concentration of CCl_4 induced hepatic damage
*P < 0.05 compared with 5% Tween 20 (vehicle)

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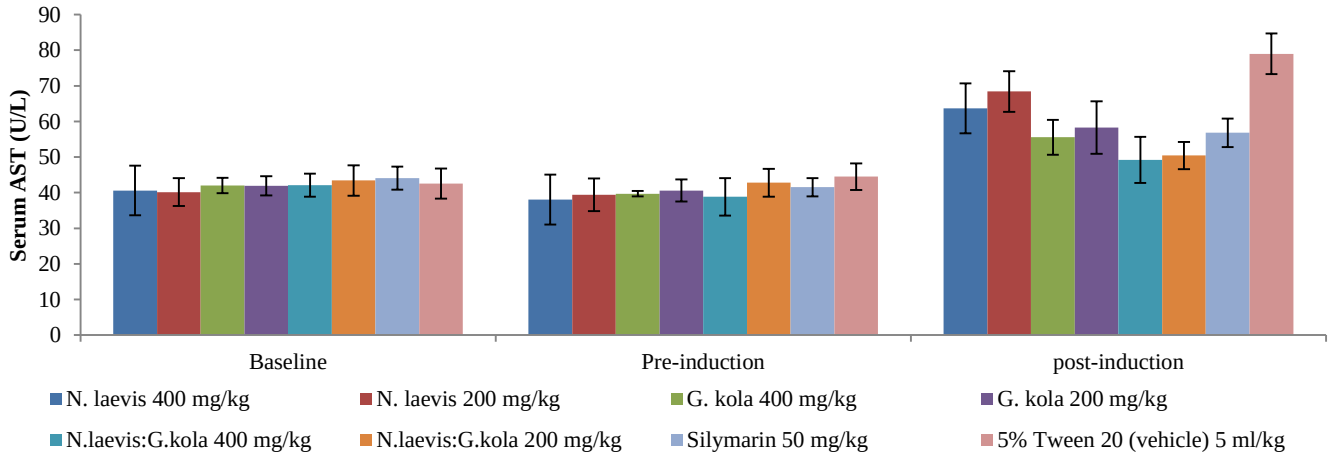

Figure 10: Effect of the extracts of *N. laevis* and G. *kola* on serum AST concentration of CCl_4 induced hepatic damage. *P <0.05 compared with 5% Tween 20 (vehicle)

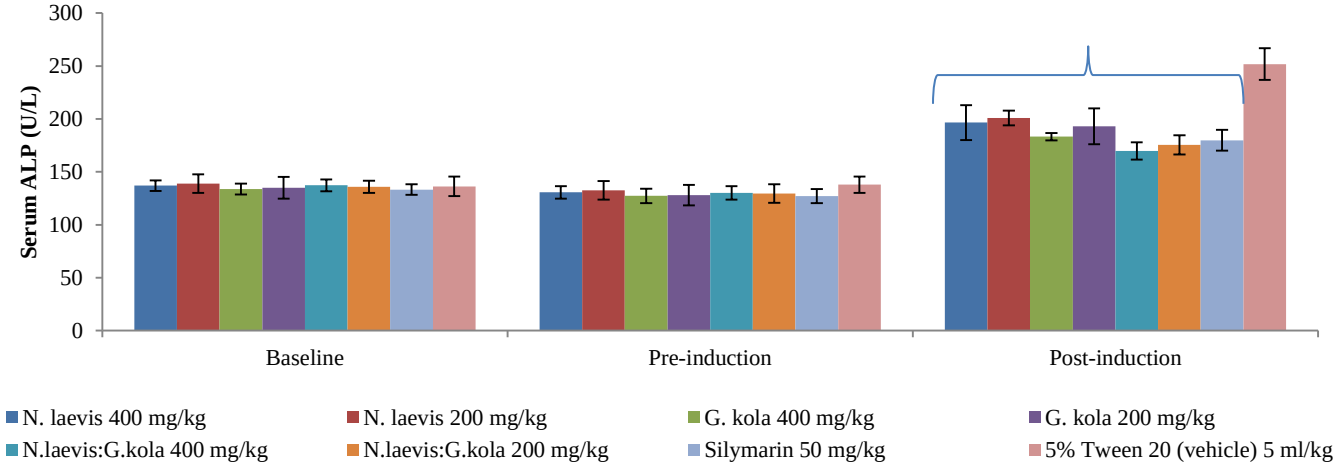

Figure 11: Effect of the extracts of *N. laevis* and *G. kola* on serum ALP concentration of CCl_4 induced hepatic damage *P < 0.05 compared with 5% Tween 20 (vehicle)

Conclusion

G. Kola and *N. laevis* showed antidiabetic and hepatorotective activities. The combination of both extracts produced reduction of blood glucose and protection against oxidative liver damage with higher synergism at lower concentration.

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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