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Newbouldia laevis on the metabolic
activities of α -glucosidase and α -
amylase**

Inhibitory effect of leaf extract of *Newbouldia laevis* on the metabolic activities of α -glucosidase and α -amylase

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Abstract

In this study, the effect of ethanol extract of the leaves of *Newbouldia laevis* on the activity of α -amylase and α -glucosidase was investigated. Inhibitory effect of *N. laevis* extract on α -glucosidase was tested *in vitro* using baker's yeast α -glucosidase and rat intestinal α -glucosidase while α -amylase inhibitory effect was assayed using rat pancreatic α -amylase. α -Glucosidase inhibitory effect of the extract was also tested *in vivo* in diabetic and non-diabetic rats. *N. laevis* extract exhibited good α -glucosidase inhibitory activity *in vitro* with IC₅₀ values of 2.2 μ g/mL and 43.5 μ g/mL for baker's yeast and rat intestinal α -glucosidase respectively. The extract also inhibited rat pancreatic α -amylase activity with IC₅₀ value of 58.7 μ g/mL. In both diabetic and non-diabetic rats, *N. laevis* extract caused a significant reduction in postprandial blood glucose level after oral sucrose load. The results of this study indicate that *N. laevis* extract exerts its glucose-lowering effect through inhibition of α -glucosidase and α -amylase.

Introduction

Diabetes mellitus is a metabolic disorder which affects millions of people around the world. It is one of the leading causes of blindness, cardiovascular complications and end-stage renal failure that results in dialysis and kidney transplantation. Diabetes is characterized by disturbance in glucose homeostasis which results in chronic hyperglycemia. This occurs when there is defect in insulin secretion and/or insulin action (Kumar et al., 2012). Autoimmunity, obesity, high carbohydrate diet and sedentary life style are the major causes of diabetes (Patel et al., 2008). Postprandial hyperglycemia has been widely reported to be the key feature of impaired glucose tolerance and early diabetes (Tentolouris et al., 2007). Therefore, one of the important therapeutic interventions employed in the management of diabetes mellitus is targeted at decreasing postprandial blood glucose level (Alexander-Lindo et al., 2007). This can be achieved by reducing the digestion of carbohydrate and absorption of glucose using inhibitors of enzymes that

are involved in the process of digestion. Inhibitors of α -amylase and α -glucosidase have been effectively used to achieve good glycemic control in diabetic patients (Lelono and Tachibana, 2013).

α -Glucosidase inhibitors are also beneficial in decreasing postprandial plasma insulin level and improving insulin sensitivity (Shinozaki et al., 1996; Breuer, 2003). Examples of α -glucosidase and α -amylase inhibitors that are currently in clinical use are acarbose, voglibose and miglitol. In spite of their therapeutic benefits, these drugs induce some adverse effects such as abdominal discomfort, bloating, flatulence and diarrhea. These effects have been attributed to excessive enzyme inhibition which results in abnormal fermentation of undigested carbohydrate by intestinal bacteria (Bischoff, 1994). Researchers are therefore interested in discovering more enzyme inhibitors with less side-effect. In addition, many antidiabetic drugs are not readily available to diabetic patients in developing nations and rural areas due to



economic hardship and other hindrances. Majority of these people depend on herbal remedy (Kolawole et al., 2012). Therefore it is necessary to search for new inhibitors of α -amylase and α -glucosidase especially from natural sources such as medicinal plants. This will be of great assistance to those who have little or no access to synthetic drugs to care for their health needs. The search may also result in the discovery of lead molecules for the development of new antidiabetic drugs.

One of the medicinal plants that are widely used in the management of diabetes mellitus across African countries is *Newbouldia laevis* (P. Beauv). It is an angiosperm which belongs to the Bignoniaceae family. Its common names are 'Fertility tree' and 'African border tree'. In Nigeria, it is known by various names among different ethnic groups. It is called 'Akoko' in Yoruba, 'Aduruku' in Hausa and 'Ogirisí' in Igbo. The extract of the leaves has been reported to lower blood glucose level in diabetic rats (Owolabi et al., 2011). In the present study, we investigated the effects of ethanol extract of *N. laevis* leaves on α -amylase and α -glucosidase in diabetic rats.

Materials and Methods

Drugs and chemicals: Streptozotocin, rat intestinal acetone powder, baker's yeast, rat pancreatic amylase, p-nitrophenyl α -D-glucopyranoside (pNPG), acarbose and sucrose were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

Preparation of plant extract: Leaves of *Newbouldia laevis* were collected from the premises of College of Health Sciences, Ladoke Akintola University of Technology, Mercyland, Osogbo Campus, Nigeria. The leaves were identified and authenticated by a taxonomist in Forest Research Institute of Nigeria (FRIN) and a voucher specimen was deposited in the herbarium of the institute (voucher specimen no: FHI 107753). The leaves were thoroughly washed with distilled water to remove soil and other debris that may contaminate the plant sample. The washed sample was then air-dried under shade in the laboratory for 5 days and then pulverized using an electric grinding machine. The powder sample (500 g) was extracted with 80% ethanol at 70°C by continuous hot percolation using a Soxhlet apparatus. The extraction was carried out for 24 hours and the resulting ethanol extract (NLet) was concentrated at 40°C in a rotary evaporator. The solid sample obtained weighed 47.5 g (yield = 9.5%). The crude ethanol extract was kept in air-tight container and stored in a refrigerator at 4°C until the time of use.

Experimental animal: Male Wistar rats weighing 180-200 g were obtained from the Animal Holding Unit of the Department of Pharmacology and Therapeutics,

Ladoke Akintola University of Technology (LAUTECH), Nigeria. The animals were housed in polypropylene cages inside a well-ventilated room. The animals were maintained under standard laboratory conditions of temperature ($22 \pm 2^\circ\text{C}$), relative humidity (55-65%) and 12 hours light/dark cycle. They were allowed to acclimatize for 2 weeks before the experiment. During the experimental period, animals were fed with a standard balanced commercial pellet diet (Ladokun Feeds Ltd. Ibadan, Nigeria) and potable tap water *ad libitum*.

Ethical consideration: All experimental procedures were conducted in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH, 1985) as well as Ethical Guidelines for the Use of Laboratory Animals in LAUTECH, Nigeria.

Induction of diabetes in experimental animal: Experimental diabetes was induced in rats which had fasted for 12 hours by a single intravenous injection of a freshly prepared solution of streptozotocin (STZ) (60 mg/kg body weight) dissolved in 0.1 M cold citrate buffer, pH 4.5 (Chen et al., 2005). The rats were allowed to drink 5% glucose solution overnight to overcome drug-induced hypoglycemia. Estimation of fasting blood glucose (FBG) was done 72 hours after injection of STZ to confirm induction of diabetes and then on the 7th day to investigate the stability of diabetic condition. Fasting blood glucose was estimated by One Touch® glucometer (Lifescan, Inc. 1995 Milpas, California, USA). Blood sample for the FBG determination was obtained from the tail vein of the rats and those with blood glucose value ≥ 200 mg/dL were selected for the study.

Baker's yeast α -glucosidase inhibition assay: The method of Shibano et al. (1997) was adapted for the assay of α -glucosidase of baker's yeast (*Saccharomyces cerevisiae*). The reaction mixture consisted of 50 μL of 0.1 M phosphate buffer (pH 7.0), 25 μL of 0.5 mM pNPG dissolved in 0.1 M phosphate buffer, pH 7.0), 10 μL of extract (20 $\mu\text{g}/\text{mL}$) and 25 μL of α -glucosidase solution. This reaction mixture was then incubated at 37°C for 30 min. The reaction was terminated by the addition of 100 μL of 0.2 M sodium carbonate solution. The enzymatic hydrolysis of the substrate was monitored spectrophotometrically at 400 nm based on the amount of p-nitrophenol released in the reaction mixture. The inhibition percentage of α -glucosidase was calculated by the following formula:

% Inhibition = $100 - \frac{\text{mean enzyme activity in sample}}{\text{mean enzyme activity in control}} \times 100$

IC₅₀ values were determined from dose-response curve of percentage inhibition versus extract concentration and compared with the IC₅₀ of acarbose (standard drug) determined under similar conditions. All experiments were carried out in triplicate.

Rat intestinal α -glucosidase inhibition assay: The method of Kim et al. (2005) was adapted for the assay of rat intestinal α -glucosidase. Rat intestinal acetone powder (0.5 g) was suspended in 10 mL of 0.9% saline, and the suspension was sonicated for 30 sec at 4°C. After centrifugation at 10,000x g, 4°C and for 30 min, the resulting supernatant was used for the assay. The reaction mixture consisted of 50 μ L of 0.1 M phosphate buffer (pH 7.0), 30 μ L of 0.5 mM pNPG (dissolved in 0.1 M phosphate buffer, pH 7.0), 10 μ L of plant extract (20 μ g/mL) and 15 μ L of rat intestinal α -glucosidase solution. This reaction mixture was then incubated at 37°C for 30 min. The reaction was terminated by the addition of 100 μ L of 0.2 M sodium carbonate solution. The α -glucosidase activity was determined by measuring the yellow colored p-nitrophenol released from pNPG at 400 nm. Percentage inhibition was calculated by the following formula:

%Inhibition = 100 - %reaction (where %reaction = mean enzyme activity in sample/mean enzyme activity in control x 100).

IC₅₀ values were determined from dose-response curve of percentage inhibition versus extract concentration and compared with the IC₅₀ of acarbose determined under similar conditions. All experiments were carried out in triplicate.

Effect of *N. laevis* extract on blood glucose level after oral sucrose load: This experiment was performed in normal and diabetic rats to confirm α -glucosidase inhibitory activity of *N. laevis* extract. Prior to the experiment, all the rats were made to fast for 12 hours. Distilled water (control), a reference drug, acarbose (50 mg/kg body weight) or NLet (500 mg/kg body weight) were orally administered to different groups of 6 rats each. Thirty minutes later, sucrose (2 g/kg body weight) was orally administered to each rat with a feeding syringe (Sancheti et al., 2011). Blood samples were collected from the tail vein by tail milking at -30 min (just before the administration of extracts and acarbose), 0 (just before the oral administration of sucrose), 60, 120, and 180 min after sucrose load. Blood glucose level was determined by glucose oxidase/peroxidase method.

α -Amylase inhibition assay: The method of Giancarlo et al. (2006) was adapted in the assay of rat pancreatic α -amylase activity. The starch substrate was prepared by dissolving 1 g of starch in 100 mL of 0.1M phosphate buffer (pH 7.0). Concentrated HCl and 0.5N NaOH were used to adjust the pH. The dinitrosalicylic acid (DNS) reagent was prepared by dissolving 5 g of 3, 5-dinitrosalicylic acid and 150 g of sodium potassium tartarate in 150 mL of distilled water and 200 mL of 1N sodium hydroxide. The mixture was refluxed in a water bath at 60°C until all components were totally dissolved and then made up to 500 mL with distilled water. Rat pancreatic amylase was dissolved in ice-cold 20 mM phosphate buffer (pH 6.7) containing 6.7 mM sodium

chloride to give a concentration of 5 units/mL. Test tubes were prepared in duplicates including blank and control. In each test tube, 1 mL of the plant extract (20 μ g/mL) and 1 mL enzyme solution were mixed and incubated at 25°C for 30 min. After incubation, 250 μ L of the starch preparation was transferred into each test tube to start the reaction. The mixture was vortexed and then incubated at 37°C for 15 min. Two millilitres of DNS (color reagent) was added and the mixture was stirred in a vortex and boiled in a water bath at 100°C for 10 min. Thereafter the mixture was cooled down in a running tap water and the absorbance was read at 540 nm using a spectrophotometer. The percentage inhibition of α -amylase was calculated by the following formula:

%Inhibition = 100 - %reaction (where %reaction = mean enzyme activity in sample/mean enzyme activity in control x 100).

The percentage inhibition of α -amylase activity was plotted against the sample concentration and a linear regression curve was established in order to calculate the IC₅₀ value.

Statistical analysis: Data were recorded as mean \pm standard error of mean (SEM) and analyzed using one-way ANOVA followed by Student's t-test. Results were considered significant at p<0.05.

Results

NLet exerted good inhibitory effect on baker's yeast α -glucosidase (Figure 1). The concentration of the extract required to cause 50%inhibition (IC₅₀) against α -glucosidase was 2.2 μ g/mL. Acarbose which was used as positive control, exerted lower α -glucosidase inhibitory potential with IC₅₀ value of 3.8 μ g/mL. NLet also showed potent inhibitory effect against rat intestinal α -glucosidase (Figure 2). The IC₅₀ value of the extract against rat intestinal α -glucosidase was 43.5 μ g/mL while that of acarbose was 62.7 μ g/mL. Figure 3 shows the effect of NLet and acarbose on blood glucose level in normal rats after sucrose administration. Thirty minutes after sucrose load, blood glucose in all the groups increased rapidly and reached a peak at 60 min. Thereafter, it gradually decreased. NLet caused a significant reduction (p<0.05) in blood glucose level at 60, 120 and 180 min compared to normal control. In diabetic rats, NLet also caused significant decrease in blood glucose at 60, 120 and 180 min (p<0.05) compared to the diabetic control (Figure 4). The reduction in blood glucose in NLet-treated diabetic rats was significantly different (p<0.05) compared to the acarbose-treated group. *In vitro* α -amylase inhibitory study demonstrated that NLet inhibited rat pancreatic α -amylase activities (Figure 5). The IC₅₀ value for NLet was 58.7 μ g/mL and that of acarbose was 92.3 μ g/mL.

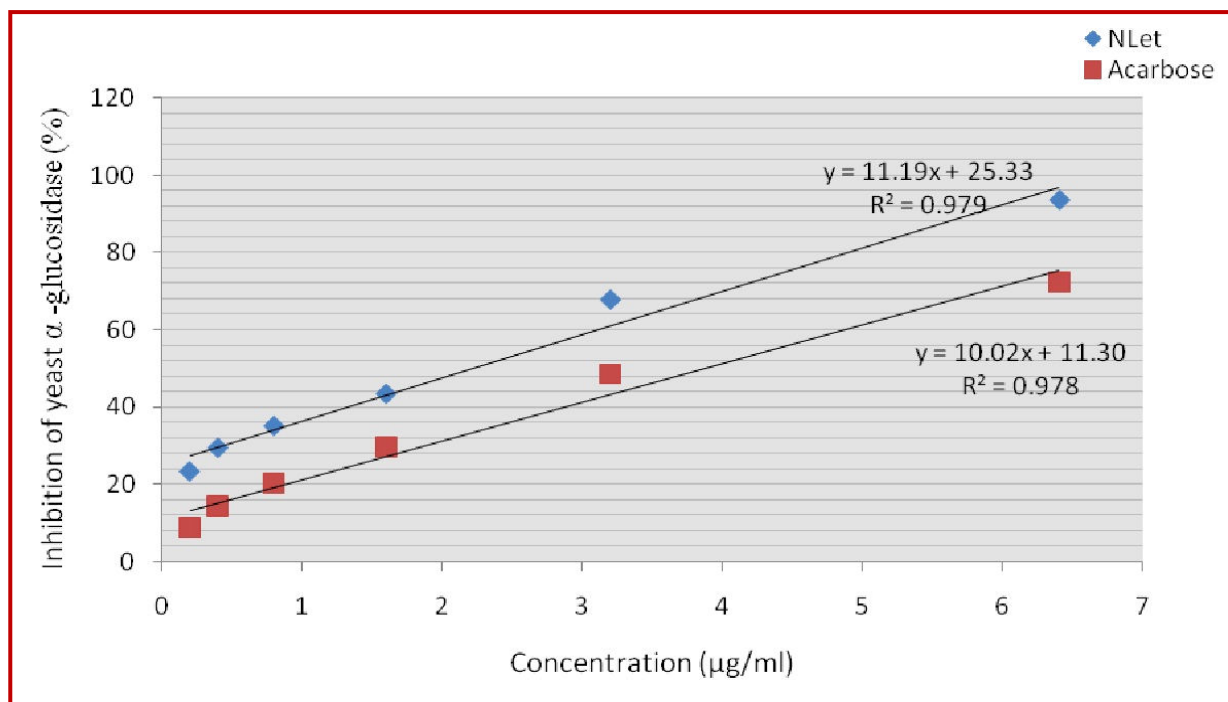


Figure 1: Inhibitory effect of *N. laevis* extract on baker's yeast α -glucosidase
Values represent mean \pm SEM of three replicates

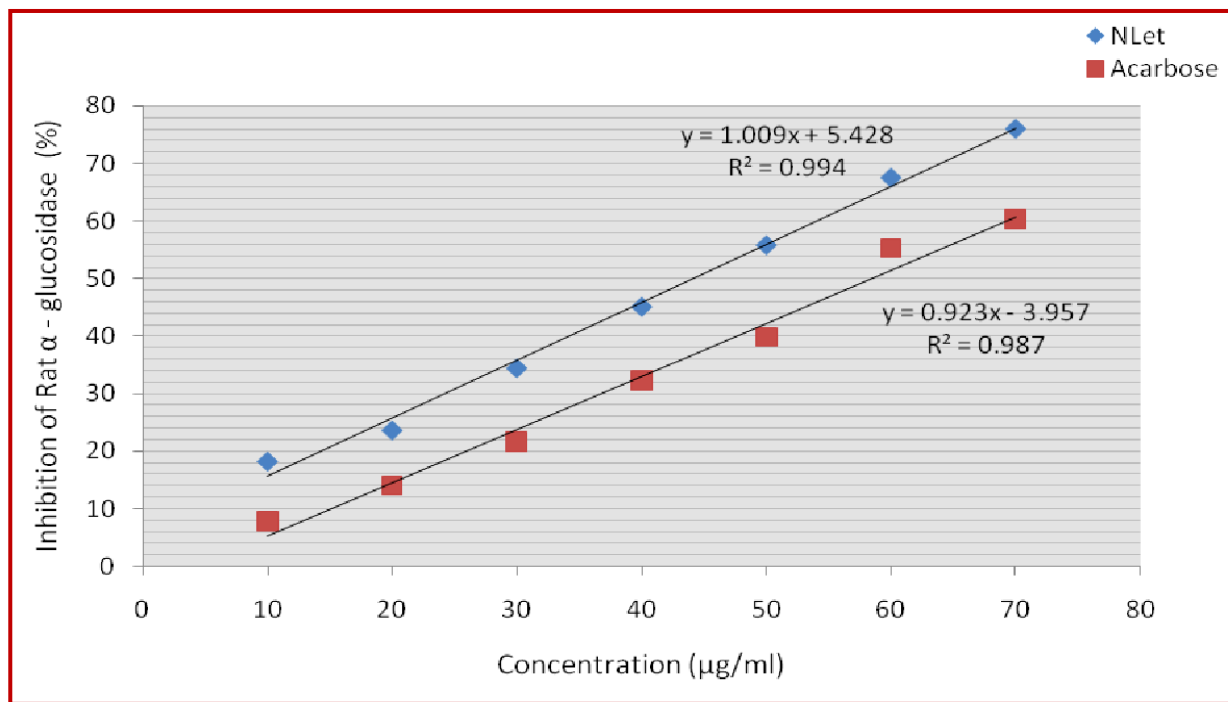


Figure 2: Inhibitory effect of *N. laevis* extract on rat intestinal α -glucosidase
Values represent mean \pm SEM of three replicates

Discussion

One of the important goals in the treatment of diabetes is to maintain fasting and postprandial blood glucose near normal levels. The suppression of production or absorption of glucose from the gastrointestinal tract

through inhibition of α -amylase and α -glucosidase enzymes is one therapeutic approach that has been successfully employed to achieve this goal (Cheng and Fantus, 2005; Bhandari et al., 2008). Polysaccharides and disaccharides are digested to produce monosaccharides

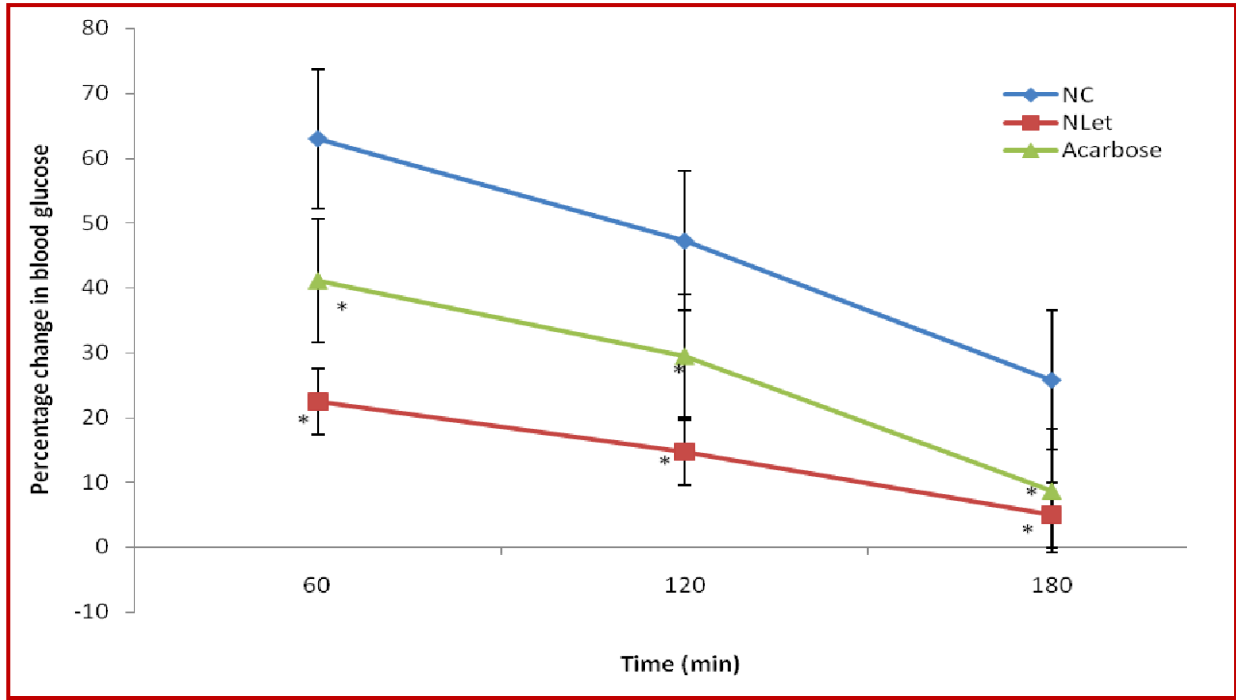


Figure 3: Effect of *N. laevis* extract on blood glucose level in normal rats after oral sucrose load
 Values are expressed as the mean \pm SEM (n = 6). *p<0.05 compared to control. NLet = ethanol extract of *N. laevis*; NC = normal control.

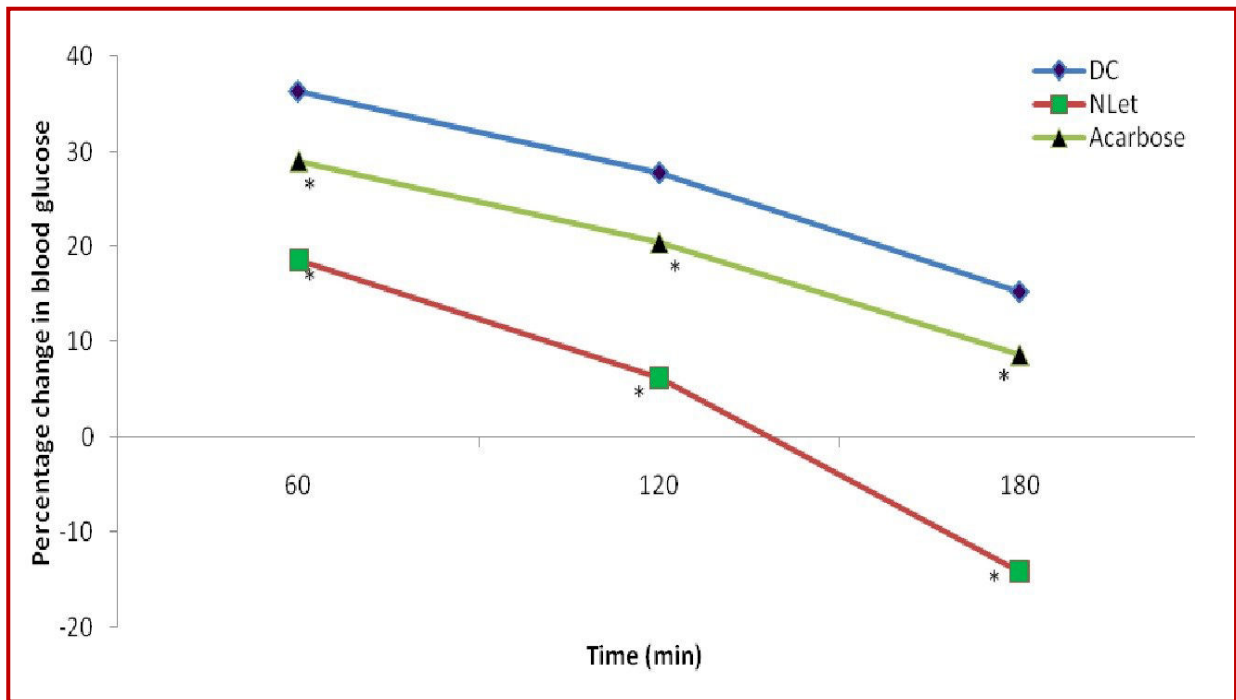


Figure 4: Effect of *N. laevis* extract on blood glucose level in diabetic rats after oral sucrose load
 Values are expressed as the mean \pm SEM (n = 6). *p<0.05 compared to diabetic control. NLet = ethanolic extract of *N. laevis*; DC = diabetic control

by these carbohydrate-hydrolyzing enzymes. α -Amylase is secreted by the salivary glands in the mouth where digestion of carbohydrate begins. However, the acidic medium of the stomach halts this process. In the upper part of the small intestine, digestion of carbohydrate continues with α -amylase secreted by the

pancreas. Oligosaccharides and disaccharides which are the products of α -amylase metabolic activity are then broken down to monosaccharides such as glucose and galactose by α -glucosidase enzymes which include maltase, isomaltase, glucoamylase and sucrase (Akintunde and Oboh, 2012). These enzymes are located in the

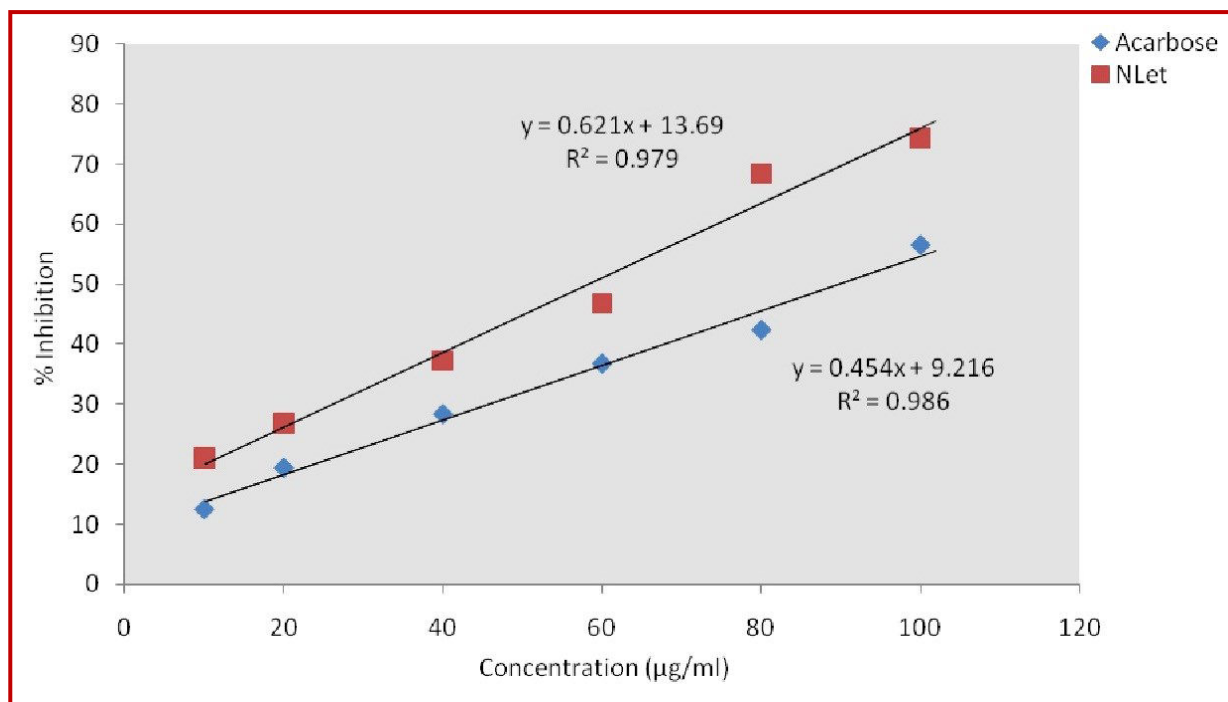


Figure 5: Inhibitory effect of *N. laevis* extract on rat pancreatic α -amylase
Values represent mean \pm SEM of three replicates

brush border of intestinal mucosa and their digestive activity results in a sharp rise in the level of blood glucose after the consumption of a carbohydrate-rich diet (Tentolouris et al., 2007).

Preclinical as well as clinical studies have shown that α -glucosidase and α -amylase inhibitors can suppress the production and absorption of glucose from the small intestine without causing hypoglycemia (Matsui et al., 2007). They are also beneficial in decreasing postprandial plasma insulin level and improving insulin sensitivity (Breuer, 2003). Acarbose and miglitol are examples of enzyme inhibitors that are already in clinical use. Some medicinal plants have also been reported to inhibit the activity of α -amylase and α -glucosidase (Dimo et al., 2007). Examples of such plants are *Musa paradisiacal* (Shodehinde and Oboh, 2012), *Tournefortia hartwegiana* (Ortiz et al., 2007) and *Allium* spp. (Nickavar and Yousefian, 2009). Secondary metabolites such as flavonoids, tannins, saponins and alkaloids present in these plants are generally believed to be responsible for their enzyme inhibition activity (Mahesh and Menon, 2004).

In the current study, the results of *in vitro* assays showed that extract of *N. laevis* inhibited the activities of α -amylase and α -glucosidase but the inhibitory effect against α -glucosidase was more pronounced. The results also showed that NLet produced better inhibitory effects than acarbose. Attempt was made in this study to confirm the observed *in vitro* inhibitory effect of *N. laevis* leaf extract on α -glucosidase through *in vivo* study by determining postprandial glucose

levels in normal and streptozotocin-induced diabetic rats after oral administration of sucrose. Sucrose needs to be digested to glucose before it is absorbed into the systemic circulation. Administration of *N. laevis* leaf extract 30 min before oral sucrose load significantly suppressed postprandial glucose level in both normal and diabetic rats. This indicates that the extract inhibited the catabolic activity of α -amylase and α -glucosidase. Therefore the enzyme inhibitor present in the leaves of *N. laevis* is a promising therapeutic remedy for the management of postprandial hyperglycemia in diabetic patients. As far as we know, this is the first time the inhibitory activity of the leaves of *N. laevis* against α -amylase and α -glucosidase is reported.

Conclusion

This study indicates that ethanol extract of the leaves of *N. laevis* exerts its glucose-lowering effect through inhibition of α -amylase and intestinal brush border α -glucosidase. Further study is necessary to isolate the active compound of the plant that is responsible for its enzyme inhibition activity and determine its structure and toxicity profile.

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