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The Effects of Aqueous and Ethanolic Extracts of *Vitex doniana* Leaf on Postprandial Blood Sugar Concentration in Wister Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OFCN designed the study, wrote the protocol and supervised the work. Author OEY carried out all laboratories work, performed the statistical analysis, managed the analyses of the study and wrote the first draft of the manuscript.

Author OFCN managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

In this study, the effects of the leaves of *V. doniana* aqueous and ethanolic extracts on postprandial blood glucose was analyzed. In the first experiment, 25 Wistar albino rats were divided into 5 groups of 5 rats each. Treatment was carried out orally for one day. Overnight fasted animals (group 2-4, 18 hrs) were orally administered different doses of the extract 10 minutes prior to the administration of sucrose at a dose of 2 g/kg. The postprandial blood sugar was measured before administration (at 0 minute), and after administration (30, and 60 minutes). In the second

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experiment, same procedure was repeated as in the first experiment but sucrose was substituted by glucose administration (2 g/kg). Results showed that both the 50 mg and 100 mg/kg ethanolic and aqueous extracts significantly decreased ($p < 0.05$) the postprandial blood sugar concentration at 30 minutes compared to the control value. Conclusively, these results suggest that ethanolic and aqueous extracts of the leaves of *Vitex doniana* have remarkable inhibitory effects on α -glucosidase enzymes with promising clinical relevance of reducing hyperglycaemia via this mechanism.

Keywords: Postprandial; blood sugar; *Vitex doniana*.

1. INTRODUCTION

α -Glucosidase inhibitors have been developed specifically to delay the digestion of complex carbohydrates and decrease the postprandial rise in plasma glucose, thus reproducing the effect of a low glycaemic index/high fibre diet [1]. These actions significantly reduced postprandial glycaemic and insulinaemic increase whether they are used as monotherapy or combined in the treatment of type 2 diabetes. These drugs have an excellent safety profile.

α -Glucosidase inhibitors competitively block small intestine brush border enzymes that are necessary to hydrolyze oligo and polysaccharides to monosaccharides [2]. Inhibition of this enzyme slows the absorption of carbohydrates and hence, the postprandial rise in plasma glucose is blunted in both normal and diabetic subjects [3]. Recent studies showed that phenolic phytochemicals from plants play an important role in modulating glucosidase and amylase activities and therefore contribute to the management of diabetes [4].

Vitex doniana sweet, (family *Verbanaceae*) is a perennial shrub widely distributed in tropical West Africa, and some East African countries including Uganda, Kenya and Tanzania; and high rainfall areas. It is found in the middle belt of Nigeria particularly Kogi, Benue, and parts of the savannah regions of Kaduna, Sokoto and Kano states [5]. It is variously called *vitex* (English), *dinya* (Hausa), *dinchi* (Gbagyi), *uchakoro* (Igbo), *oriri* (Yoruba), *ejiji* (Igala) and *olih* (Etsako) [6]. *V. doniana* is employed locally in the treatment of a variety of diseases. Hot aqueous extracts of the leaves are used in the treatment of stomach and rheumatic pains, inflammatory disorders, diarrhoea, dysentery and diabetes [5,7] indicating that the plant's leaves may possess anti-diabetic properties among others. The roots and leaves are used for nausea, colic and epilepsy [8,9]. In North-Central and eastern parts of Nigeria, the young leaves are used as vegetables or sauces and porridge for meals, especially for diabetic

patients [10]. Hence, the present study is aimed at determining its effects on postprandial blood glucose.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Fresh leaves of *Vitex Doniana* were collected from their natural habitat at Ankpa, Kogi State, and were authenticated by Mr. A.O. Ozioko in the Herbarium Unit of Department of Botany, University of Nigeria, Nsukka, where voucher specimen was deposited.

2.2 Preparation of Plant Extract

The plant material was dried in the laboratory at room temperature and pulverized using laboratory mortar and pestle.

2.2.1 Aqueous extraction

Exactly 400 g of the pulverized sample was soaked in 2 liters of distilled water (1:5 w/v) and was allowed to stand for 24 hours at room temperature after which it was filtered and the filtrate was concentrated using rotary evaporator under reduced pressure. It was allowed to dry at room temperature and stored in refrigerator prior to usage.

2.2.2 Ethanol extraction

Exactly 400 g of the pulverized sample was soaked in 2 liters (1:5 w/v) of ethanol for 24 hours. The extract was filtered under reduced pressure using filter paper, membrane filter and vacuum pump and the filtrate was concentrated using rotary evaporator under reduced pressure.

2.3 Animal Management

Male Wistar rats of average weight, 150-200 g, were purchased from the animal house of the Department of Biochemistry, Salem University, Lokoja, Nigeria. They were acclimatized for two weeks prior to commencement of experiment

and were kept at room temperature and maintained *ad libitum* on Growers mash (Vital Feed, Jos) and weighed prior to experiment.

2.4 Testing the Effects of Ethanolic/Aqueous Extracts of the Leaves after Challenge with Sucrose Solution

In the experiment, 25 Wistar albino rats were divided into 5 groups of 5 rats each. Treatment was carried out orally for one day. Overnight fasted animals (group 2-4, 18hrs) were orally administered different doses of the extract 10 minutes prior to the administration of sucrose at a dose of 2 g/kg. The postprandial blood sugar was measured before administration (at 0 minute), and after administration (30, and 60 minutes) using Accu-Check Active glucometer. The experiment was also repeated using aqueous extract.

- Group 1: Control (administered 2 g/kg sucrose, no treatment).
- Group 2: Administered 2 g/kg b.w. sucrose before treatment with 50 mg/kg b.w. ethanolic/aqueous extract.
- Group 3: Administered 2 g/kg b.w. sucrose before treatment with 100 mg/kg b.w. ethanolic/aqueous extract.
- Group 4: Administered 2 g/kg b.w. sucrose before treatment with 200 mg/kg b.w. ethanolic/aqueous extract.
- Group 5: Normal (no sucrose, no treatment).

2.5 Testing the Effects of Ethanolic/Aqueous Extracts of the Leaves after Challenge with Glucose Solution

In the experiment, a total of 25 albino rats were divided into 5 groups of 5 rats each. Treatment was carried out orally for one day. Overnight fasted animals (group 2-4) were orally administered different doses of the extract 10 minutes prior to the administration of glucose (2 g/kg). The postprandial blood sugar was measured before administration (at 0 minute), and after administration (30, and 60 minutes respectively) using Accu-Check Active glucometer. The experiment was also repeated using the aqueous extract.

- Group 1: Control (administered 2 g/kg glucose, no treatment).
- Group 2: Administered 2 g/kg glucose before treatment with 50 mg/kg aqueous/ethanolic extract.

Group 3: Administered 2 g/kg glucose before treatment with 100 mg/kg aqueous/ethanolic extract.

Group 4: Administered 2 g/kg glucose before treatment with 200 mg/kg aqueous/ethanolic extract.

Group 5: Normal (no glucose, no treatment).

2.6 Statistical Analysis

All the values estimations were expressed as mean \pm standard deviation and analyzed for ANOVA and post hoc Duncan's -test using SPSS. Differences between groups were considered significant at $p < 0.05$ levels.

3. RESULTS

3.1 Effects of Different Doses of Ethanol Extract on Postprandial Blood Sugar after Sucrose Ingestion

Postprandial blood sugar test showed a dose dependent decrease in blood sugar of ethanolic extract treated animals (Fig. 1). After 30 minutes of sucrose ingestion, the glycaemic level was between 125-127 mg/dl in the extract treated groups. Control group had 148mg/dl at 30 minutes and 133 mg/dl at 1 hour. Similarly, there was significant decrease ($P < 0.05$) in postprandial blood sugar after 1 hour in 100 mg and 200 mg/kg treated groups.

3.2 Effects of Different Doses of Aqueous Extract on Postprandial Blood Sugar after Sucrose Ingestion

Like ethanolic extract treatment (Fig. 1), similar observation was made in Fig. 2. Postprandial blood sugar test showed a dose dependent decrease in blood sugar of ethanolic(aqueous) extract treated animals (Fig. 2). Similarly, there was significant decrease ($P < 0.05$) in postprandial blood sugar after 1 hour in 100mg and 200mg/kg treated groups as shown below (Fig. 2).

3.3 Effects of Different Doses of Ethanolic Extract on Postprandial Blood Sugar after Glucose Ingestion

After 30 minutes, there was significant increase in postprandial blood sugar in the test and control groups compared to normal and also compared with zero minute (before ingestion of sugar). At 1 hour (60 minutes) there was significant decrease in postprandial blood sugar in the extract treated groups in a non dose-dependent manner.

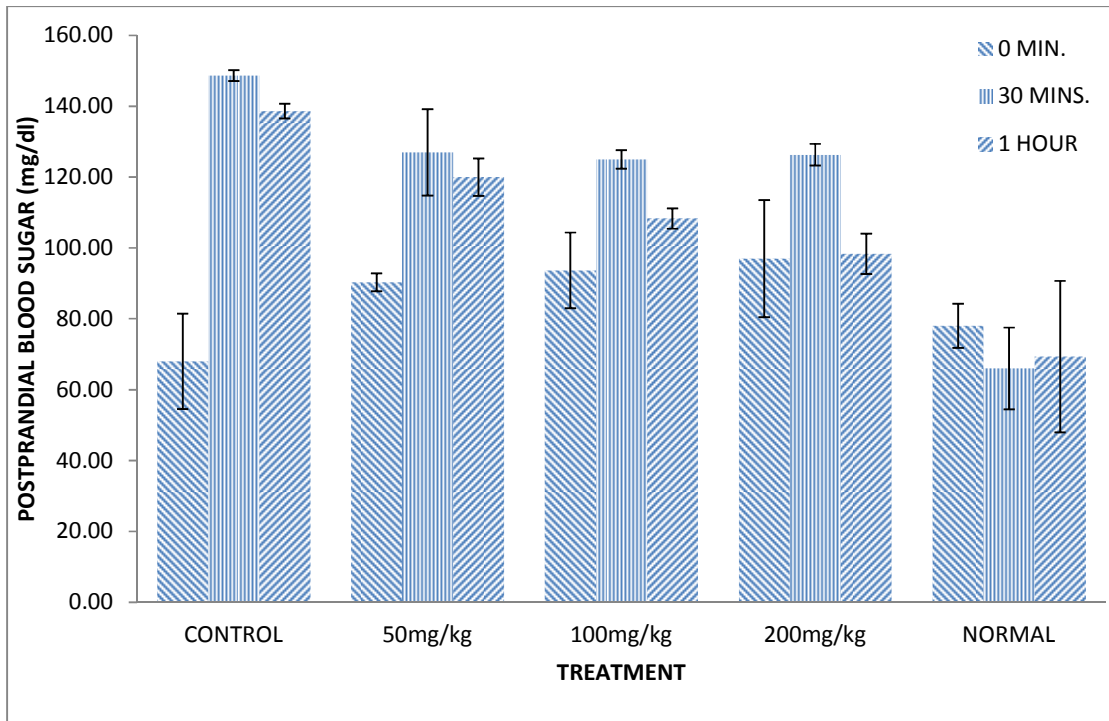


Fig. 1. Effects of different doses of ethanolic extract on postprandial blood sugar after sucrose ingestion

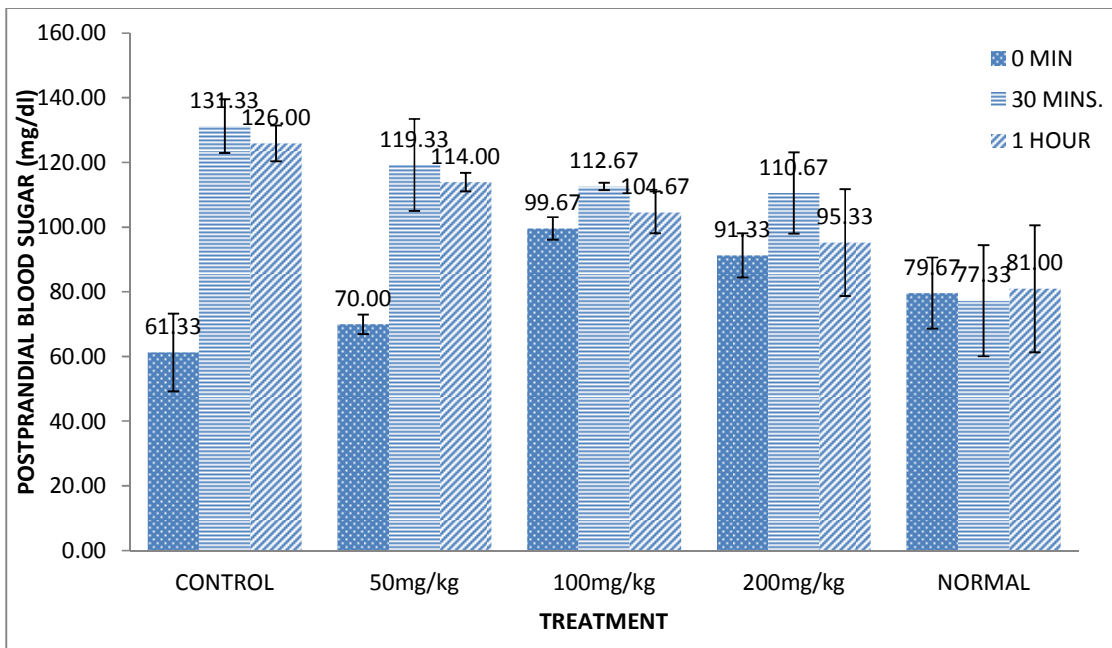


Fig. 2. Effects of different doses of aqueous extract on postprandial blood sugar after sucrose ingestion in normoglycaemic rats

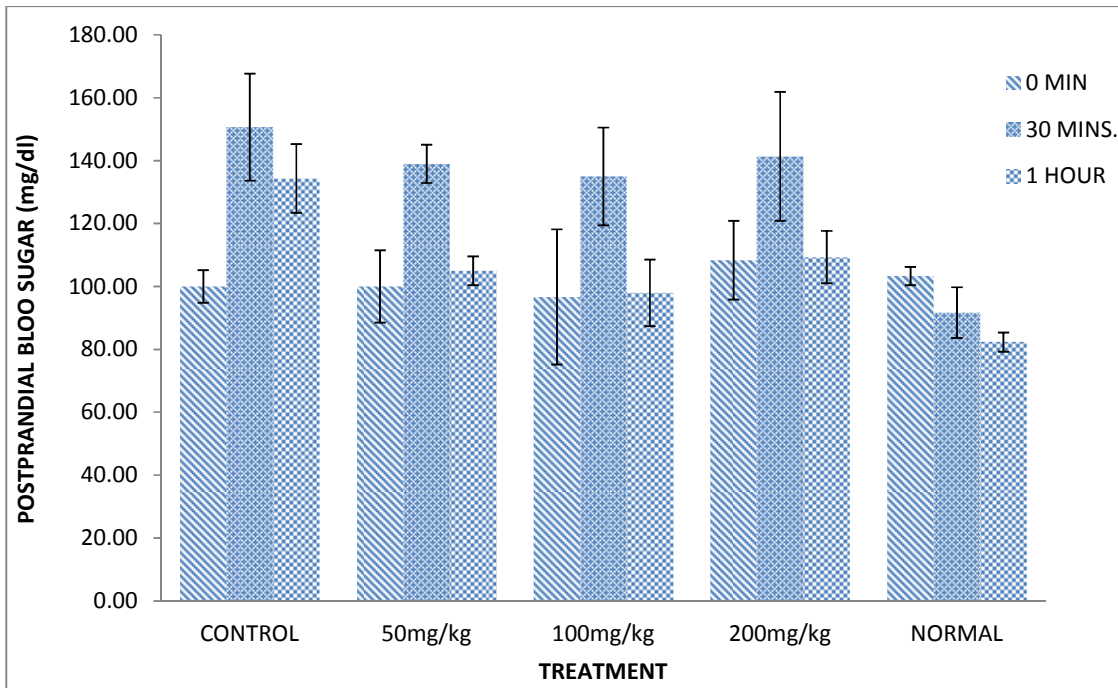


Fig. 3. Effects of different doses of ethanolic extract on postprandial blood sugar after glucose ingestion in normoglycaemic rats

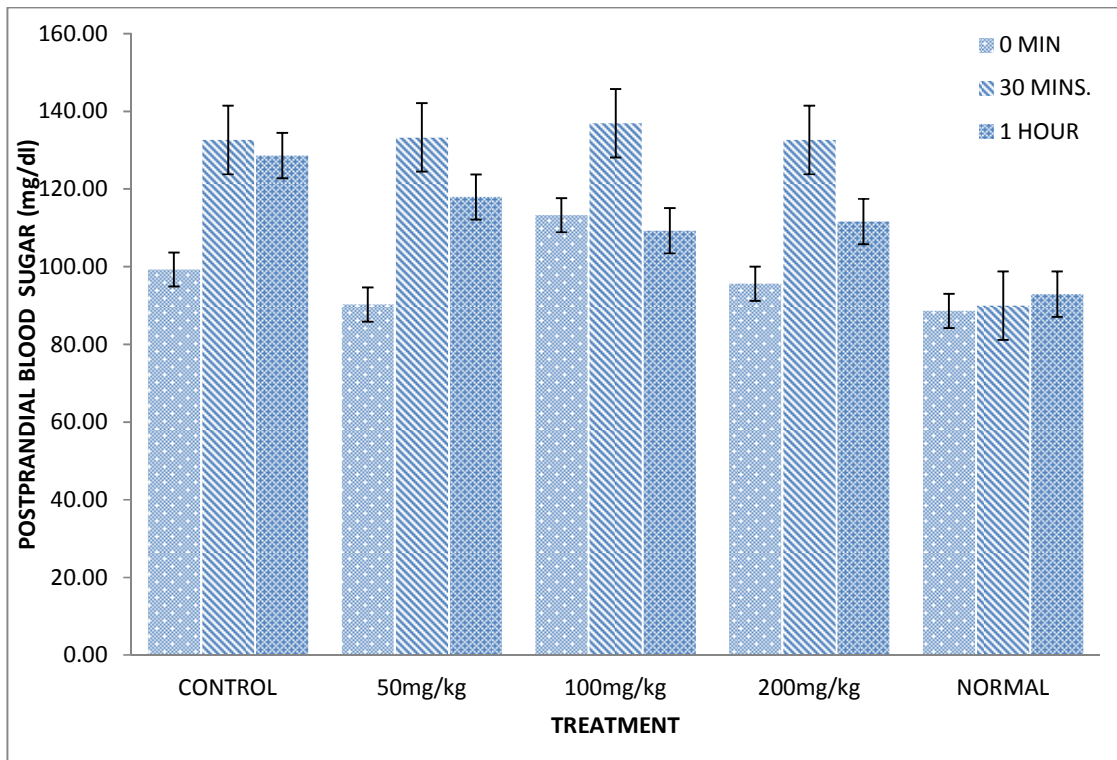


Fig. 4. Effects of different doses of aqueous extract on postprandial blood sugar after glucose ingestion

3.4 Effects of Different Doses of Aqueous Extract on Postprandial Blood Sugar after Glucose Ingestion

Like Fig. 3, similar observation was made in Fig. 4. After 30 minutes, there was significant increase in postprandial blood sugar in the test and control groups compared to normal and also compared with zero minute (before ingestion of sugar) at 1 hour (60 minutes) there was significant decrease in postprandial blood sugar in the extract treated groups in a non dose-dependent manner.

4. DISCUSSION

Many pharmacological therapies have been used to improve the status of diabetes by several mechanisms such as, inhibition of carbohydrate metabolizing enzymes, manipulation of glucose transporters, β -cell regeneration and enhancing the insulin releasing activity [11]. At present, drug therapies either alone or in combination cannot restore blood glucose homeostasis and many limitations exist in their use [12]. The management of diabetes without any side effect is still a challenge to the medical system. Many efforts have been made to identify new hypoglycaemic agents obtained from different sources especially medicinal plants because of their effectiveness, fewer side effects and relatively low cost. Several medicinal plants have been investigated for their beneficial use in different types of diabetes in the Indian traditional medicine even though their biologically active compounds and efficacy are unknown [13].

An effective strategy for diabetes management especially type 2 diabetes is the inhibition of α -amylase and α -glucosidase enzymes. Inhibition of α -amylase and α -glucosidase enzymes involved in the dietary carbohydrate digestion can significantly decrease the postprandial increase of blood glucose after a carbohydrate diet and therefore can be an important strategy in the management of postprandial blood glucose level in type 2 diabetic patients and borderline patients [14,15].

α -Glucosidases are located in the brush border surface membrane of intestine. They are the key enzymes involved in digestion of disaccharides into simpler sugars [16,17]. The α -glucosidase inhibitors could slow the liberation of glucose from oligosaccharides and disaccharides, resulting in delaying glucose absorption and

decreasing postprandial plasma glucose levels [18-20]. Hence the inhibitory potential of *Vitex doniana* was shown in Figs. 1-4, where both the aqueous and ethanolic extract treatment displayed significant ($P<0.05$) decrease in postprandial blood glucose in rats 30 minutes after sucrose ingestion compared with the control in a non-dose dependent manner. Postprandial blood glucose measured 30 minutes after ingestion of glucose using the same treatment was higher than that of sucrose ingestion. After 60 minutes of glucose ingestion, extract treated rats showed significant reduction in postprandial blood glucose. As a result, the glucose uptake suggested that *Vitex doniana* may also be acting by increasing the peripheral utilization of glucose.

5. CONCLUSION

In conclusion, the study showed that *Vitex doniana* is a promising plant for the management of diabetes, especially postprandial blood sugar concentration.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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