

Determination of the effects of aqueous and ethanol extracts of *Vitex doniana* leaf on postprandial blood sugar concentration in Wister rats

ABSTRACT

In this study, the inhibitory effects of the leaves of *V. doniana* aqueous and ethanol extracts on α -glucosidase inhibitors were analysed. 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 mg/kg. The postprandial blood sugar was measured before administration (at 0 minute), and after administration (30, and 60 minutes). The experiment was repeated using aqueous extract. In the second phase of 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 mg/kg). The postprandial blood sugar was measured before administration (at 0 minute), and after administration (30, and 60 minutes respectively). The experiment was also repeated using the aqueous extract. There was significant decrease ($p<0.05$) in the postprandial blood sugar concentration at 30 minutes using 50 mg and 100 mg/kg ethanol extract except at 200 mg/kg (117 mg/dl) opposed to control (130 mg/dl) and 105 mg/dl opposed to control (127 mg/dl) at 60 minutes. Similar observation was made using aqueous extract. However, these results suggest that ethanol 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

31 effect of a low glycaemic index/high fibre diet [1]. These actions significantly reduce
32 postprandial glycaemic and insulinaemic increase whether they are used as monotherapy or
33 combined in the treatment of type 1 and type 2 diabetes. These drugs have an excellent safety
34 profile.

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

41 *Vitex doniana* sweet, (family *Verbanaceae*) is a perennial shrub widely distributed in tropical
42 West Africa, and some East African countries including Uganda, Kenya and Tanzania; and
43 high rainfall areas. It is found in the middle belt of Nigeria particularly Kogi, Benue, and
44 parts of the savannah regions of Kaduna, Sokoto and Kano states [5]. It is variously called
45 *vitex* (English), *dinya* (Hausa), *dinchi* (Gbagyi), *ucha koro* (Igbo), *oriri* (Yoruba) *ejiji* (Igala)
46 and *olih* (Etsako) [6]. *V. doniana* is employed locally in the treatment of a variety of diseases.
47 Hot aqueous extracts of the leaves are used in the treatment of stomach and rheumatic pains,
48 inflammatory disorders, diarrhoea dysentery and diabetes [5,7] indicating that the plant's
49 leaves may possess antidiabetic properties among others. The roots and leaves are used for
50 nausea, colic and epilepsy [8,9]. In North-Central and eastern parts of Nigeria, the young
51 leaves are used as vegetables or sauces and porridge for meals, especially for diabetic patients
52 [10]. Hence, the need to determine its effects on postprandial blood glucose.

53 **2. Materials and methods**

54 **2.1 Plant collection and identification**

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

58 **2.2 Preparation of plant extract**

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

61

62 **2.2.1 Aqueous extraction**

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

67 **2.2.2 Ethanol extraction**

68 Exactly 400g of the pulverized sample was soaked in 2 litres (1:5 w/v) of ethanol/n-Hexane
69 (2:1 v/v) for 24 hours. The extract was filtered under reduced pressure using filter paper,
70 membrane filter and vacuum pump and the filtrate was concentrated using rotary evaporator
71 under reduced pressure.

72 **2.3 Animal management**

73 Male albino rats of average weight, 150-200g, were purchased from the animal house of the
74 Department of Biochemistry, Salem University, Lokoja, Nigeria. They were acclimatized for
75 two weeks prior to commencement of experiment and were kept at room temperature and
76 maintained *ad libitum* on Growers mash (Vital Feed, Jos) and weighed prior to experiment.

77 **2.4 Postprandial blood sugar test using sucrose**

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

84 Group 1: Control (administered 2 g/kg sucrose, no treatment).

85 Group 2: Administered 2 g/kg b.w. sucrose before treatment with 50 mg/kg b.w. ethanol
86 extract.

87 Group 3: Administered 2 g/kg b.w. sucrose before treatment with 100 mg/kg b.w. ethanol
88 extract.

89 Group 4: Administered 2 g/kg b.w. sucrose before treatment with 200 mg/kg b.w. ethanol
90 extract.

91 Group 5: Normal (no sucrose, no treatment)

92 **2.5 Postprandial blood sugar test using glucose solution**

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

99 Group 1: Control (administered 2 g/kg glucose, no treatment).

100 Group 2: Administered 2 g/kg glucose before treatment with 50 mg/kg aqueous extract).

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

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

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

104

105 **3. Results**

106 **3.1. Effects of different doses of ethanol extract on postprandial blood sugar after** 107 **sucrose ingestion**

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

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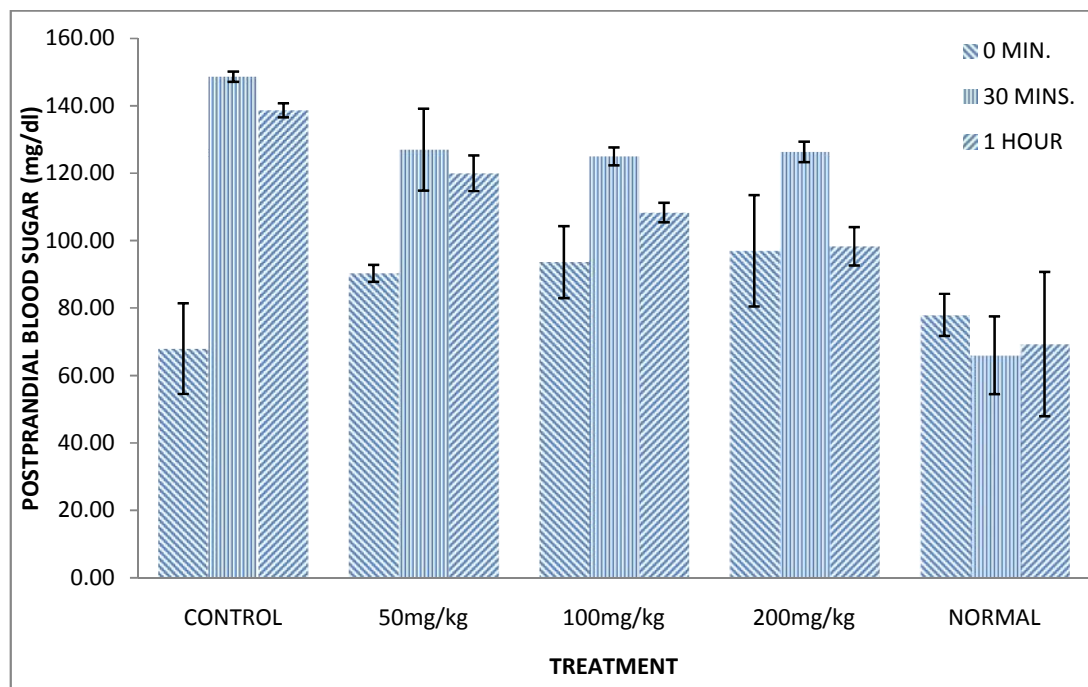


Fig. 1. Effects of different doses of ethanol extract on postprandial blood sugar after sucrose ingestion

3.2. Effects of different doses of aqueous extract on postprandial blood sugar after sucrose ingestion

Like ethanol extract treatment (figure 1), similar observation was made in figure 2. Postprandial blood sugar test showed a dose dependent decrease in blood sugar of ethanol extract treated animals (figure 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 (figure 2).

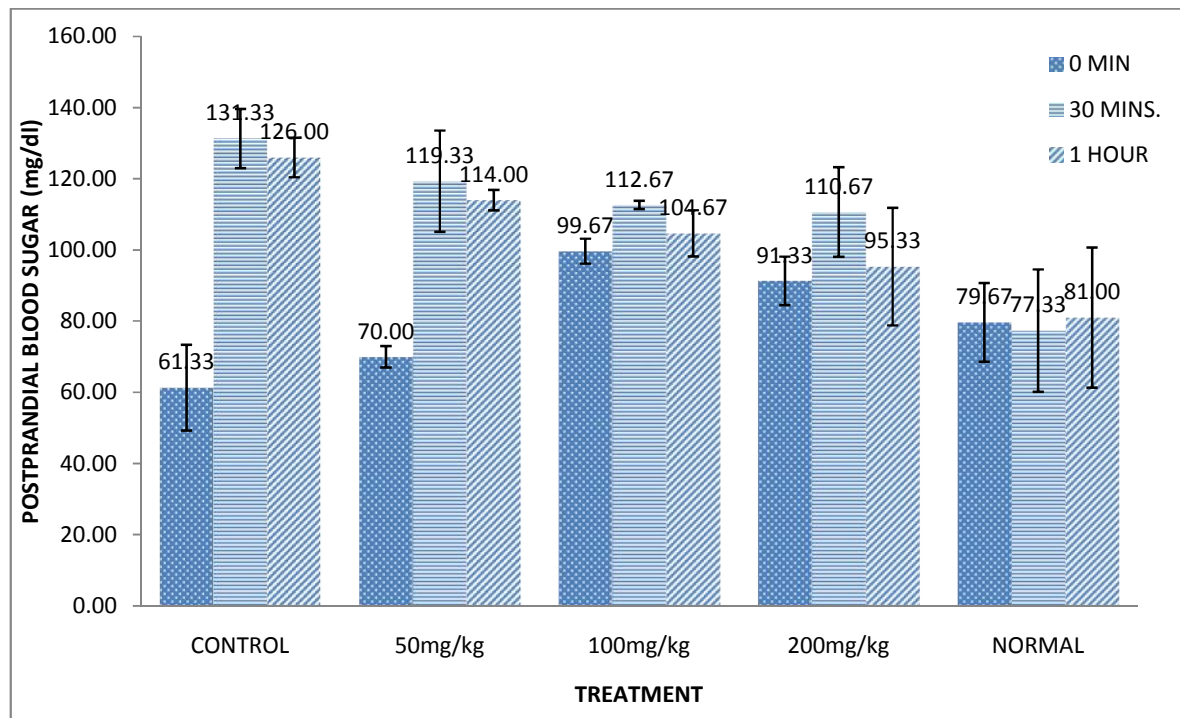


Fig.2. Effects of different doses of aqueous extract on postprandial blood sugar after sucrose ingestion in normoglycaemic rats.

3.3 Effects of different doses of ethanol 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.

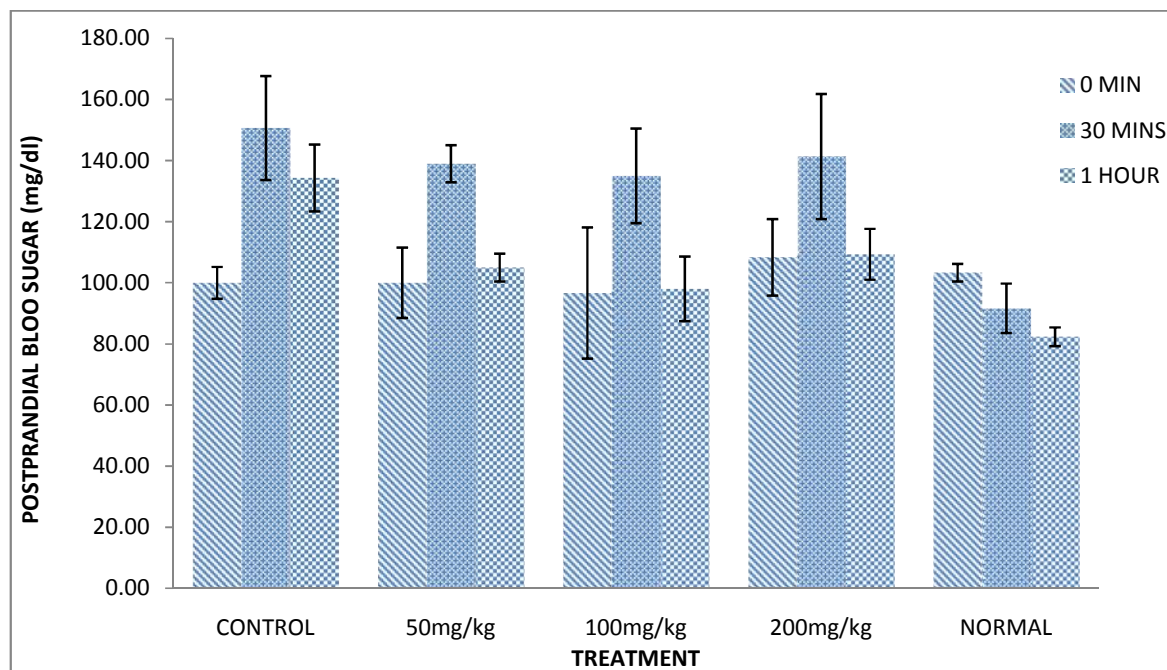


Fig.3. Effects of different doses of ethanol extract on postprandial blood sugar after glucose ingestion in normoglycaemic rats.

3.4 Effects of different doses of aqueous extract on postprandial blood sugar after glucose ingestion

Like figure 3, similar observation was made in figure 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.

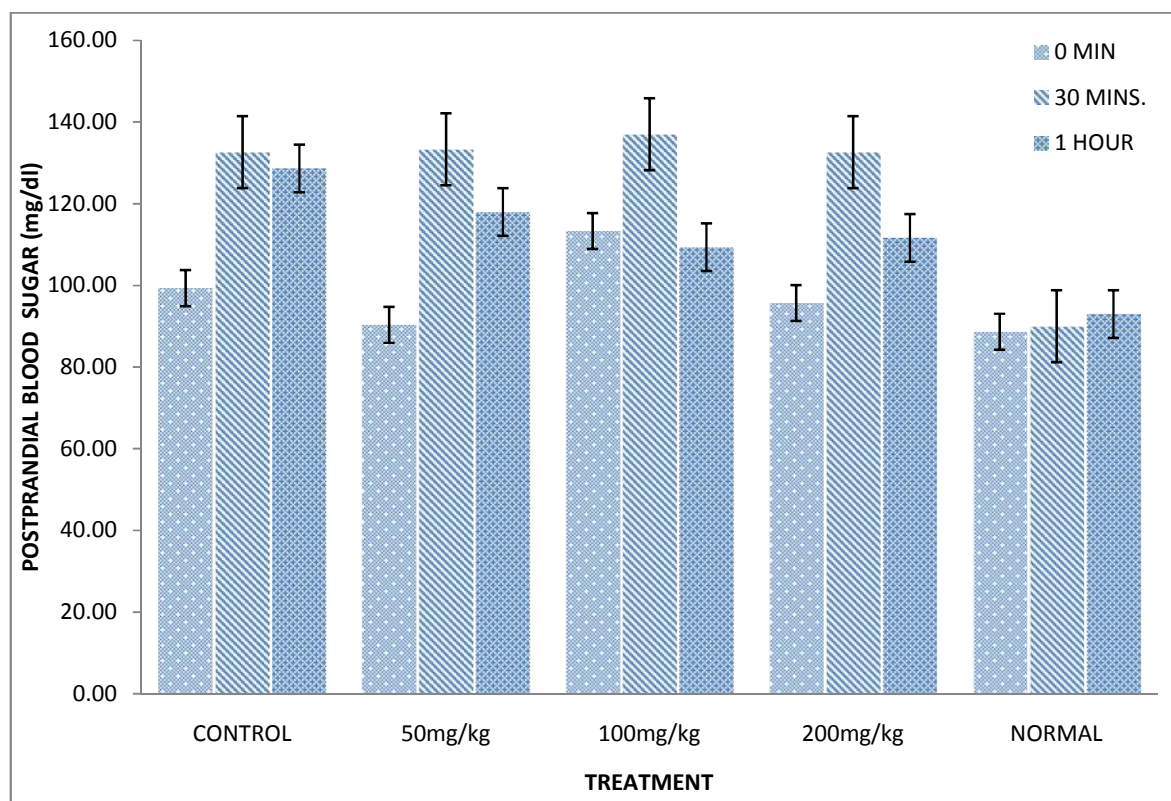


Fig.4. Effects of different doses of aqueous extract on postprandial blood sugar after glucose ingestion

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].

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 [15]. 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 [16]. Hence the inhibitory potential of *Vitex doniana* was shown in Figure 1-4, where both the aqueous and ethanol 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.

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