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Research Article
In vitro Inhibition of Carbohydrate Metabolizing Enzymes and *in vivo* Anti-hyperglycaemic Potential of Methanol Extract of *Desmodium velutinum* Leaves

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Abstract

Background and Objective: Hydrolysis of carbohydrates to dextrin by pancreatic α-amylase activity which is further hydrolyzed into glucose by intestinal α-glucosidase enzyme causes elevation of blood glucose leading to postprandial hyperglycaemia. Inhibition of these enzymes is an important strategy in the management of diabetes mellitus. The aim of this study was to evaluate the effect of methanol extract of *Desmodium velutinum* leaves (MEDVL) on α-amylase *in vitro* and a possible corresponding anti-hyperglycaemic effect *in vivo*. **Materials and Methods:** The *in vitro* assay for inhibitory effect of MEDVL on α-amylase and α-glucosidase activities was performed according to standard protocols by reacting the enzymes and substrates with different concentrations (20-100 μg mL^{-1}) of the extract in relation to acarbose while the enzyme kinetics (mode of Inhibition) assay for both enzymes were determined using the Lineweaver-Burk plot. The *in vivo* anti-hyperglycaemic activity of the extract in relation to acarbose was investigated after starch and sucrose load on alloxan induced hyperglycaemic rats. **Results:** The results revealed that MEDVL had a higher IC_{50} (88.2 and 83.5 μg mL^{-1}) than acarbose (73.5 and 73.9 μg mL^{-1}) for α-amylase and α-glucosidase, hence less potent than acarbose. The enzyme kinetics assay, the Lineweaver-Burke plot showed that MEDVL demonstrate a mixed non-competitive inhibition of α-amylase and uncompetitive inhibition of α-glucosidase activities compared with the control (acarbose) that it inhibits α-amylase in a competitive and α-glucosidase in a non-competitive manner. The *in vivo* anti-hyperglycaemic analyses revealed that the MEDVL caused significant reduction ($p<0.05$) in the postprandial blood glucose level, suppressed postprandial rise in blood glucose in rats within 2 h after starch and sucrose load. **Conclusion:** The MEDVL is a potent inhibitor for carbohydrate metabolizing enzymes which can be used in the management of type 2 diabetes mellitus.

Key words: Postprandial hyperglycaemia, diabetes mellitus, acarbose, *Desmodium velutinum*, enzyme kinetics

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mankind over the years have suffered from various kinds of epidemics that are ravaging the races in a gradual manner. Diabetes mellitus is an endocrine disease that has beguile mankind with world overall occurrence rate of 4-5%[1]. This endocrine disease is a group of metabolic disorders characterized by a chronic hyperglycaemic condition resulting from defects in insulin secretion, insulin action or both[2]. The number of adults with diabetes in 2000 was 177 million[3] and the incidence of diabetes among adults in 2010 was 285 million (6.4%) and this value is predicted to rapidly increase to around 439 million (7.7%) by 2030[4]. The hallmark of diabetes mellitus is hyperglycaemia which is solely responsible for its cardinal symptoms namely: Weight loss, polyphagia, polydipsia and polyuria[5]. The sudden rise in blood glucose levels or hyperglycaemia in type 2 diabetes is caused by hydrolysis of starch to dextrin by pancreatic α-amylase activity which is further hydrolyzed into glucose by intestinal α-glucosidase enzyme and these enzymes are well known to play essential leading roles in carbohydrate metabolism[6]. Effective strategy for the management of type 2 diabetes is the inhibition of carbohydrate hydrolyzing enzymes such as pancreatic α-amylase and intestinal α-glucosidase[7]. Inhibiting of alpha-amylase and alpha-glucosidase activities in the intestine result in decreased of postprandial elevation of plasma glucose by delaying the complete digestion and absorption of starch[8].

Many therapeutic approaches have been utilized for treatment of this disorder including the use of oral hypoglycaemic agents. Despite the availability of various orthodox medications for the control and management of hyperglycaemia, there has been increasing demand for the use of plant products with anti-diabetic activity[9].

Desmodium velutinum is a perennial, erect or semi-erect shrub or sub-shrub, up to 3 m high. Branches often dark red, yellow-brown when young, velutinous and short hooked-hairy with purple to pink colour flowers. *Desmodium velutinum* belongs to the botanical family Fabaceae which is generally called "Ikeagwuani" in Igbo and ''Dangeree'' in Hausa natives of Nigeria. *Desmodium* has been used in traditional medicine in a broad range for the treatment of diarrhea, dysentery and stomach-ache, wound, ulcers and other skin problems, stones in the gall bladder, kidney, headache, toothache, fever, rheumatism, jaundice and gonorrhea[10].

The increasing prevalence of type 2 diabetes mellitus and the negative clinical outcomes observed with the commercially available anti-diabetic drugs have led to the investigation of new therapeutic approaches focused on controlling postprandial glucose levels. The use of carbohydrate digestive enzyme inhibitors from natural sources could be a possible strategy to block dietary carbohydrate absorption with less adverse effects than synthetic drugs.

The aim of this study was to investigate the *in vitro* effect of methanol extract of *Desmodium velutinum* leaves on carbohydrate metabolizing enzymes and *in vivo* anti-hyperglycaemic effect of methanol extract of *Desmodium velutinum* leaves on alloxan induced hyperglycaemic rats challenge with starch and sucrose load.

MATERIALS AND METHODS

Collection and identification of plant material: A whole fresh plant of *Desmodium velutinum* was collected fresh from Orba-Nsukka, Enugu State. The plant was identified by Mr. Alfred Ozioko of Bioresources Development and Conservation Programme, Nsukka, Enugu State.

Research duration: The research was carried out in Biochemistry Laboratory, University of Nigeria, Nsukka for a period of six months (September, 2016-January, 2017).

Chemicals and drugs

Enzymes and substrates: *Aspergillus niger* α-amylase (EC 3.2.2.1), baker yeast α-glucosidase, soluble starch, p-nitrophenyl-α-D-glucopyranoside (Sigma-Aldrich, UK), Acarbose (Glucobay® Bayer), methanol (BDH, England), alloxan monohydrate (Sigma-Aldrich, UK), were purchased from the country representative of Sigma Chemical, St. Loius USA. A digital glucometer and corresponding test strips (Fine Test®, Infopia Co., Ltd. USA) was purchased from a pharmacy store in Nsukka Local Government Area, Enugu State. All other chemicals used were of analytical grade and obtained commercially.

Preparation of methanol leaves extract of *Desmodium velutinum*: The plant leaves were air-dried at room temperature for 14 days and pulverized with an electric hammer mill. Cold maceration of pulverized plant leaves was extracted with methanol (98% v/v) with occasional shaking for 48 h. The mixture was filtered and the filtrates were pooled, concentrated and evaporated to dryness over a water bath at 45°C. The extract will hence forth be referred to as MEDVL (methanol extract of *Desmodium velutinum* leaves) and was then stored in a refrigerator until required for use.

Enzyme inhibition protocol

Assay of alpha-amylase inhibition: Assay of alpha-amylase inhibition was investigated according to the method of McCue and Shetty[11], with slight modification. Volume of 250 µL of the plant extract of various concentrations (20-100 µg mL^{-1}) was placed in a test tube and 250 µL of α-amylase solution (0.5 mg mL^{-1}) in 0.02M sodium phosphate buffer (pH 6.9) was added. The test tube solution was pre-incubated for 10 min at 25°C, after which 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing 1% starch solution was added at timed intervals and then incubated at 25°C for 10 min. Then, 500 µL of 3,5-Dinitrosalicylic acid (DNS) reagent was added to terminate the reaction. The tubes were cooled at room temperature after incubating in boiling water for 5 min. Then, 5 mL of distilled water was added to the reaction mixture to dilute it and absorbance was measured at 540 nm using a spectrophotometer. The control and blank were prepared using the same procedure replacing the extract with Dimethyl Sulfoxide (DMSO) and distilled water, respectively. The percentage inhibition of α-amylase inhibitory activity will be calculated as, thus:

$$\text{Inhibition (\%)} = \frac{\Delta A_{control} - \Delta A_{extract}}{\Delta A_{control}} \times 100$$

Where:

$\Delta A_{control} = A_{control} - A_{blank}$

$\Delta A_{extract} = A_{extract} - A_{blank}$

Then 50% inhibitions of enzyme activity (IC_{50}) of the extract concentration were determined graphically.

Alpha-amylase mode of inhibition: The type of inhibition was investigated according to the modified method described by Ali *et al.*[12], 250 µL of α-amylase solution was pre-incubated with 250 µL(5 mg mL^{-1}) of the extract at 25°C for 10 min in one set of tubes. In another set of tubes, 250 µL of phosphate buffer (pH 6.9) was pre-incubated with α-amylase. Then, 250 µL of starch solution was added to both sets of reaction mixtures to start the reaction at increasing concentrations (0.30-5.0 mg mL^{-1}). The reaction mixture was then incubated at 25°C for 10 min and then boiled for 5 min after addition of 500 µL of DNS to stop the reaction. The rate of reducing sugars released was spectrophotometrically determined using a maltose standard curve and converted to reaction velocities. A double reciprocal plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted. The type of inhibition of the crude extract on α-amylase activity was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

Assay of alpha-glucosidase inhibition: The assay method described by Apostolidis *et al.*[13], with slight modification was used to determine α-glucosidase inhibitory activity. About 100 µL of α-glucosidase solution was incubated with 50 µL of the extract of varying concentrations (20-100 µg mL^{-1}) at 25°C for 10 min followed by the addition of 50 µL of 5.0 mM p-nitrophenyl-α-D-glucopyranoside (P-NPG) solution in 0.1M phosphate buffer (pH 6.9). The reaction mixture was then incubated for 5 min at 25°C and 2 mL of 0.1 M Na_2CO_3 was added to stop the reaction. The α-glucosidase inhibitory activity was determined spectrophotometrically at 405 nm by measuring the rate of the yellow coloured p-nitrophenol released from pNPG. The control and blank were prepared using the same method by replacing the extract with DMSO and distilled water, respectively. The α-glucosidase inhibitory activity was calculated by using the following formula:

$$\text{Inhibition (\%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

The 50% inhibitory capacity (IC_{50}) values of the extract concentration was determine from plots of percent inhibition versus concentration.

Mode of α-glucosidase inhibition: The method described by Ali *et al.*[12] was used to determined mode of inhibition of α-glucosidase. The 100 µL of α-glucosidase solution (0.5 mg mL^{-1}) with 50 µL (5 mg mL^{-1}) of the extract were pre-incubated at 25°C for 10 min in one set of tubes. In another set of tubes, 50 µL of phosphate buffer (pH 6.9) and α-glucosidase was pre-incubated. Then, 50 µL of pNPG at increasing concentrations (0.63-2.0 mg mL^{-1}) was added to both sets of reaction mixtures to start the reaction. The reaction mixture was then incubated for 10 min and 500 µL of Na_2CO_3 was added to stop the reaction. The amount of reducing sugars released was spectrophotometrically at 405 nm determined using a p-nitrophenol standard curve and converted to reaction velocities. A double reciprocal (Lineweaver-Burk) plot (1/v versus 1/[S]) where v is reaction velocity and [S] is substrate concentration was plotted. The type of α-glucosidase inhibitory activity by the crude extract was determined by analysis of the double reciprocal (Lineweaver-Burk) plot using Michaelis-Menten kinetics.

Starch tolerance test: The starch tolerance test was conducted using the method described by Mogale *et al.*[14] with slight modification. Twenty four rats divided into six groups of four animals each were used for the study. Group I will serve as the diabetic controls (DC). After an overnight fast (18 h), group I rats received 1 mL distilled water. Groups II and III rats received 400 and 800 mg kg^{-1} MEDV, respectively. Groups IV and V rats were treated with these same doses (400 and 800 mg kg^{-1}, respectively) but co-administered with a fixed dose of acarbose (150 mg kg^{-1} b.wt.). Rats in group VI were treated with 150 mg kg^{-1} acarbose body weight only. Twenty minutes after treatment, each rat received an oral starch load (3 g kg^{-1}). Post-prandial blood glucose levels were measured before (0 min) and at 30, 60, 90 and 120 min, after oral administration of starch. Post-prandial blood glucose of experimental rats was compared with those of diabetic control rats.

Sucrose tolerance test: Four days after the starch tolerance test experiment, sucrose tolerance tests for the same group of rats used for the starch tolerance test were performed. The experimental protocol for performing the sucrose tolerance test was similar to the one used in the starch tolerance tests except that instead of starch, sucrose (5 g kg^{-1} body mass) was orally administrated to all groups of rats, 20 min after administration of the plant extract.

Statistical analysis: Data expressed as Mean±SD were analyzed using the Statistical Package for Social Sciences (SPSS), version 20. The data were analyzed by one way analysis of variance (ANOVA) followed by Duncan *post-hoc* test. Differences of $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Adequate management of blood glucose level is a critical strategy in the control of diabetes and its complications[15]. Glycemic control in diabetes is largely dependent on effective management of postprandial glucose[16]. The α-glucosidase and α-amylase inhibition play a major role in the therapeutic approaches as oral hypoglycemic drugs for control of hyperglycaemia especially in patients with type 2 diabetes mellitus and borderline patients[17]. Therefore, screening of these two enzymes in the plant has received more attention for delay carbohydrate digestion and prolongs overall carbohydrates digestion time leading to a reduction in the rate of glucose absorption and consequently decreasing postprandial plasma glucose rise[18].

In the present study, Table 1 and 2 reveals that concentration-dependent inhibitory effect on α-amylase and α-glucosidase enzymes was observed at various concentrations of the extract and acarbose (standard drug) used. The median Inhibitory Concentration (IC_{50}) of the MEDVL on α-amylase and α-glucosidase was estimated to be 88.2 and 83.5 μg mL^{-1}, while that of the standard acarbose was 73.5 and 73.9 μg mL^{-1}. The low percentage inhibition (Table 1 and 2) implies that the methanol extract of *Desmodium velutinum* leaves are very potent α-amylase and α-glucosidase inhibitor in comparison with acarbose and served a natural source of antidiabetic drug. Any plant with strong inhibitor for

Table 1: Percentage inhibitory effect of various concentrations of methanol extract *Desmodium velutinum* leaves (MEDVL) and acarbose (AC) on the activity of α-amylase enzyme

Plant extract	Concentration (μg mL^{-1})	Inhibition (%)	IC_{50} (μg mL^{-1})	Standard drug	Concentration (μg mL^{-1})	Inhibition (%)	IC_{50} (μg mL^{-1})
Desmodium	20	12.40±0.66	88.2	Acarbose	20	18.76±1.14	73.5
velutinum	40	23.98±0.41			40	26.35±0.32	
	60	35.56±0.32			60	46.37±0.66	
	80	47.74±0.15			80	51.45±0.91	
	100	51.62±0.35			100	63.57±0.67	

Data represented as Mean±S.D. Data were analyzed by one-way ANOVA followed by Duncan's *post- hoc* test for multiple comparisons (n = 3)

Table 2: Percentage inhibitory effect of various concentration of methanol extract *Desmodium velutinum* leaves (MEDVL) and acarbose (AC) on the activity of α-glucosidase enzyme

Plant extract	Concentration (μg mL^{-1})	Inhibition (%)	IC_{50} (μg mL^{-1})	Standard drug	Concentration (μg mL^{-1})	Inhibition (%)	IC_{50} (μg mL^{-1})
Desmodium	20	16.97±0.27	83.5	Acarbose	20	20.28±0.55	73.9
velutinum	40	25.03±0.27			40	28.77±0.22	
	60	38.31±0.97			60	41.82±0.33	
	80	47.19±0.22			80	52.74±0.63	
	100	65.98±0.17			100	70.22±0.28	

Data represented as Mean±S.D. Data were analyzed by one-way ANOVA followed by Duncan's *post-hoc* test for multiple comparisons (n = 3)

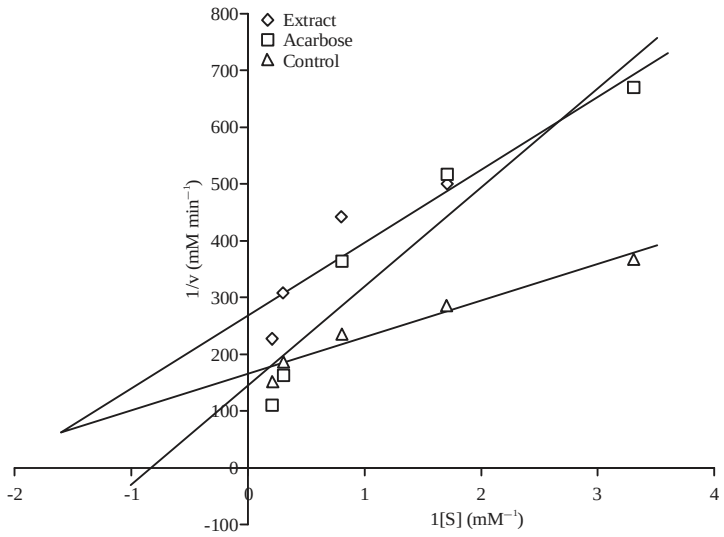

Fig. 1: Lineweaver-Burk plots for the investigation of mode of inhibition of α-amylase activity by methanol extract of *Desmodium velutinum* leaves (MEDVL) and acarbose (standard drug)

α-amylase and α-glucosidase could serve as effective therapy for postprandial hyperglycaemia with minimal side effects[19]. This is also in agreement with the report of Pinto *et al.*[20], which stated that dietary management of hyperglycaemia linked to diabetes mellitus can be targeted through foods or botanical supplements that have moderate α-amylase inhibition.

In order to ascertain the mode of inhibition of α-amylase and α-glucosidase, further study was carried out using methanol extract of *Desmodium velutinum* leaves (MEDVL) and acarbose. The mode of inhibition of the enzyme was generated using Lineweaver-Burke plot and the result showed that MEDVL inhibited α-amylase in a mixed non-competitive manner with increasing K_m of 0.391-0.476 mM and there is a reduction in V_{max} of 6.05×10^{-3}-3.72×10^{-3} mM min^{-1} (Fig. 1). This suggests that the inhibitor binds exclusively to the free enzyme yielding an enzyme-inhibitor complex. The binding of the inhibitor to the free enzyme decrease the apparent affinity of the enzyme for the substrate (K_m value appears to increase, decreasing V_{max}). The active component in the extract is structurally not similar to the substrate, rather the extract binds reversibly to the site other than active site of the enzyme and occupies it in a mutually exclusive manner that causes conformational changes in the enzyme that reduce the affinity of the enzyme for its substrate[21]. The manner of competitive inhibition of α-amylase exhibited by acarbose with increasing K_m of 0.391-1.19 mM with a relatively constant V_{max} of 6.05×10^{-3}-6.83×10^{-3} mM min^{-1} (Fig. 1), indicates that the active component is structurally similar to the substrate. This implies that the acarbose compete for the active site of the enzyme and occupies it in an exclusive manner with the substrate[22].

The inhibition kinetics of methanol extract of *Desmodium velutinum* leaves analyzed by Lineweaver-Burk plot indicates that MEDVL inhibited α-glucosidase in an uncompetitive manner with reduction in K_m of 31.69-2.53 mM and also reduction in V_{max} of 1.78×10^{-1}-1.45×10^{-2} mM min^{-1} (Fig. 2). This suggests that the extract has no structural similarity with the substrate and do not compete for the active site of the enzyme with the substrate rather the inhibitor within the extract binds exclusively to the enzyme-substrate complex yielding an inactive enzyme-substrate-inhibitor complex[22]. Therefore, MEDVL decreases the K_m of α-glucosidase because of increased binding efficiency and decrease V_{max} of α-glucosidase because they interfere with substrate binding and hamper catalysis in the ES complex. The inhibition kinetics of acarbose analyzed by Lineweaver-Burk plot indicates that it inhibited α-glucosidase in a non-competitive manner with reduction of K_m (31.68-12.72 mM) and a reduction of V_{max} (1.78×10^{-1}-5.0×10^{-2} mM min^{-1}) (Fig. 2). This suggests that the acarbose has no structural similarity with the substrate and do not compete for the active site of the enzyme with the substrate, the acarbose binds exclusively to the enzyme-substrate complex yielding an inactive enzyme-substrate inhibitor complex. The different inhibition kinetics observed between α-amylase and α-glucosidase by MEDVL and acarbose could be due to structural differences related to the origins of the enzymes[15]. The inhibitory effects of MEDVL on α-amylase and α-glucosidase activities,

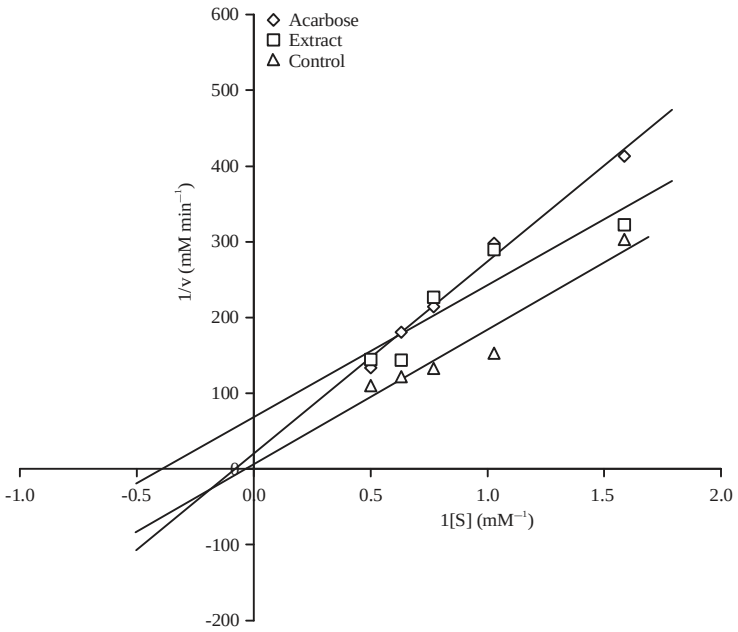

Fig 2: Lineweaver-Burk plots for the investigation of mode of inhibition of α-glucosidase activity by methanol extract of *Desmodium velutinum* leaves (MEDVL) and acarbose (standard drug)

may be attributed to the presence of phytochemicals such as flavonoids, tannins and saponins. This is attributed to phytochemical analyses carried out by Ezike *et al.*[23], revealed that methanol extract of *Desmodium velutinum* contains flavonoids, alkaloids, steroids, terpenoids and resins. This could also be justified by the nature of some extract constituents (phenols, flavonoids, saponins, steroids, alkaloids, terpenoids) which could be responsible as being effective inhibitors of α-amylase and α-glucosidase[24]. The retardation and delay of carbohydrate absorption with a plant-based α-amylase and α-glucosidase inhibitor offers a prospective therapeutic approach for the management of type 2 diabetes mellitus.

In order to ascertain the *in vitro* anti-hyperglycaemic or hypoglycaemic effect of the methanol extract of *D. velutinum* leaves *, in vivo* investigation of the effect of the *D. velutinum* extract on postprandial hyperglycaemic diabetic rats challenged with oral administration of potato starch and sucrose was tested. The administration of an oral starch load, rapidly increased blood glucose levels in all the various hyperglycaemic groups within 30 min (Fig. 3).The mean blood glucose levels of treated group 2 (400 mg kg^{-1} MEDVL) exhibited statistically non-significant ($p<0.05$) difference in blood glucose level within 60 min compared with group I (diabetic control), but showed a statistically significant ($p<0.05$) decrease of blood glucose level at 90 and 120 min when compared to the group I (diabetic control). The mean blood glucose levels of treated group III (800 mg kg^{-1} MEDVL) showed a statistically significant ($p<0.05$) decrease at 60, 90 and 120 min when compared with group I (diabetic control), also exhibited statistically significant ($p<0.05$) decrease at 90 and 120 min when compared with group II (Fig. 3).

The mean blood glucose levels of the extract MEDVL alongside with acarbose (400 mg kg^{-1} MEDVL/150 mg kg^{-1} AC, 800 mg kg^{-1} MEDVL/150 mg kg^{-1} AC) and acarbose (150 mg kg^{-1}) treated group showed a statistically significant ($p<0.05$) decrease in blood glucose at 60, 90 and 120 min when compared to group I (diabetic control) (Fig. 3).

The same group of animals after 4 days were challenged with sucrose load and there was a rapid increased in blood glucose level in all the various hyperglycaemic groups within 30 min of administration of an oral sucrose load (Fig. 4). All the diabetic treated groups such as group II (400 mg kg^{-1} MEDVL), group III (800 mg kg^{-1} MEDVL), the extract MEDVL alongside with acarbose group IV (400 mg kg^{-1} MEDVL/150 mg kg^{-1} AC), group V (800 mg kg^{-1} MEDVL/150 mg kg^{-1} AC) and acarbose group VI (150 mg kg^{-1} AC) showed a statistically significant ($p<0.05$) decrease in blood glucose at 60, 90 and 120 min when compared to group 1 (diabetic control) (Fig. 4). The results from the starch and sucrose tolerance reveals that oral administration of methanol extract of *Desmodium velutinum* to rats fed with both starch and sucrose significantly

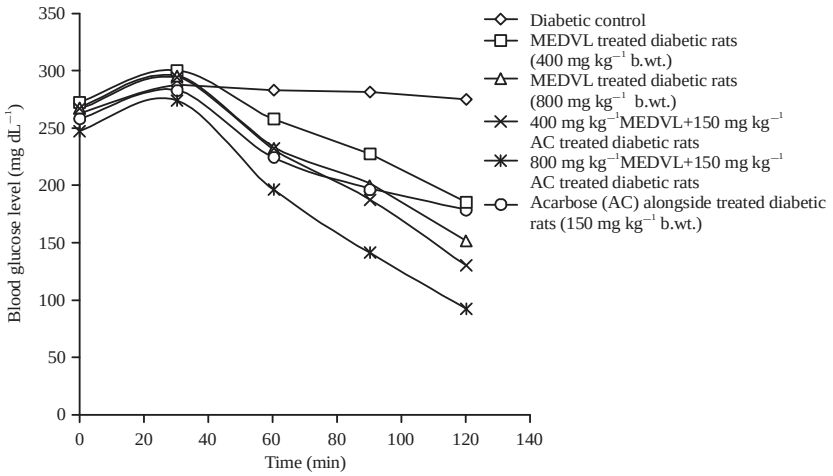

Fig. 3: Effect of methanol extract of *Desmodium velutinum* leaves (MEDVL), acarbose (AC) and MEDVL alongside acarbose administration on oral starch tolerance of hyperglycaemic rats

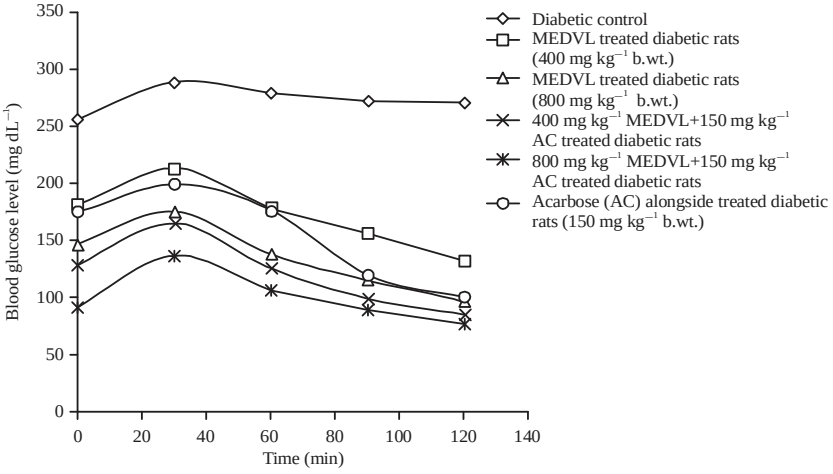

Fig. 4: Effect of methanol extract of *Desmodium velutinum* leaves (MEDVL), acarbose (AC) and MEDVL alongside acarbose administration on oral sucrose tolerance of hyperglycaemic rats

suppressed the rise in postprandial hyperglycaemia in all the diabetic treated rats, whereas administration of distilled water to diabetic control rats did not affect the rise in postprandial hyperglycaemia. However, oral co-administration of the *Desmodium velutinum* extract with acarbose and acarbose alone treated groups to 18 h fasted diabetic rats 20 min before oral administration of starch and sucrose significantly suppressed the rise in postprandial hyperglycaemia at 60, 90 and 120 min in these rats. Therefore, the reduction in the blood glucose level by the *Desmodium velutinumm* ethanol extract treated rats compared to the diabetic control animals suggest that the methanol extract of the *Desmodium velutinum* leaves slowed down the digestion and absorption of starch and sucrose in the animals. This study is also in agreement with the study of Mogale *et al.*[14], where *Sclerocarya birrea* stem-bark hexane extracts significantly suppressed the rise in postprandial glucose level after oral administration of sucrose but failed to replicate similar effects after oral administration of starch and glucose in both normal and diabetic rats.

CONCLUSION

Findings from this study have demonstrated the antidiabetic potential of *Desmodium velutinum via* its *in vitro* inhibitory action on both α-amylase and α-glucosidase and

significantly reduce the rise of postprandial hyperglycaemic in diabetic rats loaded with starch and sucrose *in vivo*. Therefore, *Desmodium velutinum* leaves extract could be potentially used as natural therapy for type 2 diabetes mellitus with minimal side effects.

SIGNIFICANCE STATEMENT

This research proved the use of *Desmodium velutinum* as a source of significant plant based inhibitor for carbohydrate metabolizing enzymes, a possible strategy to block dietary carbohydrate absorption. Therefore, it could open new therapeutic approach focused on controlling postprandial glucose levels in the management of type 2 diabetes with less adverse effect than synthetic drugs.

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REFERENCES

1. Koyuturk, M., O. Ozsoy-Sacan, S. Bolkent and R. Yanardag, 2005. Effect of glurenorm on immunohistochemical changes in pancreatic β cells of rats in experimental diabetes. Indian J. Exp. Biol., 43: 268-271.
2. Khan, A., G. Zaman and R.A. Anderson, 2009. Bay leaves improve glucose and lipid profile of people with type 2 diabetes. J. Clin. Biochem. Nutr., 44: 52-56.
3. WHO., 1999. Definition, diagnosis and classification of diabetes mellitus and its complications. Report of Word Health Organization Consultation. Geneva, Switzerland, pp: 66.
4. Shaw, J.E., R.A. Sicree and P.Z. Zimmet, 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res. Clin. Pract., 87: 4-14.
5. Cootney, R.W., 2001. Ultrasound imaging: Principles and applications in rodent research. Ilar J., 42: 233-247.
6. Bolen, S., L. Feldman, J. Vassy, L. Wilson, H.C. Yeh and S. Marinopoulos *et al*., 2007. Systematic review: Comparative effectiveness and safety of oral medications for type 2 diabetes mellitus. Ann. Int. Med., 147: 386-399.
7. Qin, X., L. Ren, X. Yang, F. Bai and L. Wang *et al*., 2011. Structures of human pancreatic α-amylase in complex with acarviostatins: Implications for drug design against type II diabetes. J. Struct. Biol., 174: 196-202.
8. Krentz, A.J. and C.J. Bailey, 2005. Oral antidiabetic agents: Current role in type 2 diabetes mellitus. Drugs, 65: 385-411.
9. Mukherjee, P.K., K. Maiti, K. Mukherjee and P.J. Houghton, 2006. Leads from Indian medicinal plants with hypoglycemic potentials. J. Ethnopharmacol., 106: 1-28.
10. Anowi, C.F., A.F. Onyegbule, E. Onyekaba and C. Azode, 2012. Antipyretic and phytochemical evaluation of the ethanol extract of the leaves of *Desmodium velutinum*. Asian J. Pharm. Life Sci., 2: 135-143.
11. McCue, P.P. and K. Shetty, 2004. Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase *in vitro*. Asia Pac. J. Clin. Nutr., 13: 101-106.
12. Ali, H., P.J. Houghton and A. Soumyanath, 2006. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes, with particular reference to *Phyllanthus amarus*. J. Ethnopharmacol., 107: 449-455.
13. Apostolidis, E., Y.I. Kwon and K. Shetty, 2007. Inhibitory potential of herb, fruit and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Innov. Food Sci. Emerg. Technol., 8: 46-54.
14. Mogale, M.A., S.L. Lebelo, N. Thovhogi, A.N. de Freitas and L.J. Shai, 2013. α-Amylase and α-glucosidase inhibitory effects of *Sclerocarya birrea* [(A. Rich.) Hochst.] subspecies *caffra* (Sond) Kokwaro (Anacardiaceae) stem-bark extracts. Afr. J. Biotechnol., 10: 15033-15039.
15. Di Carli, M.F., J. Janises, J. Ager and G. Grunberger, 2003. Role of chronic hyperglycemia in the pathogenesis of coronary microvascular dysfunction in diabetes. J. Am. Coll. Cardiol., 41: 1387-1393.
16. Parkin, C.G. and N. Brooks, 2002. Is postprandial glucose control important? Is it practical in primary care settings? Clin. Diabetes, 20: 71-76.
17. Karthic, K., K.S. Kirthiram, S. Sadasivam, B. Thayumanavan and T. Palvannan, 2008. Identification of α-amylase inhibitors from *Syzygium cumini* Linn seeds. Indian J. Exp. Biol., 46: 677-680.
18. Kimura, Y., Y. Araki, A. Takenaka and K. Igarashi, 2006. Protective effect of dietary nasunin and parapect induced oxidative stress in rat. Biosci. Biotechnol. Biochem., 63: 799-804.
19. Kwon, Y.I., D.A. Vattem and K. Shetty, 2006. Evaluation of clonal herbs of *Lamiaceae* species for management of diabetes and hypertension. Asia Pac. J. Clin. Nutr., 15: 107-118.
20. Pinto Mda, S., L.G. Ranilla, E. Apostolidis, F.M. Lajolo, M.I. Genovese and K. Shetty, 2009. Evaluation of antihyperglycemia and antihypertension potential of native Peruvian fruits using *in vitro* models. J. Med. Food, 12: 278-291.

21. Mayur, B., S. Sandesh, S. Shruti and S. Sung-Yum, 2010. Antioxidant and α-glucosidase inhibitory properties of *Carpesium abrotanoides* L. J. Med. Plant Res., 4: 1547-1553.
22. Kazeem, M.I., O.G. Raimi, R.M. Balogun and A.L. Ogundajo, 2013. Comparative study on the α-amylase and α-glucosidase inhibitory potential of different extracts of *Blighia Sapida* Koenig. Am. J. Res. Commun., 1: 178-192.
23. Ezike, A.C., P.A. Akah, C.O. Okoli and I.K. Ufere *et al*., 2014. Studies on gastrointestinal effects of *desmodium velutinum*: A traditional remedy for diarrhea. Am. J. Pharmacol. Toxicol., 9: 114-124.
24. Kim, M.H., S.H. Jo, H.D. Jang, M.S. Lee and Y.I. Kwon, 2010. Antioxidant activity and α-glucosidase inhibitory potential of onion (*Allium cepa* L.) extracts. Food Sci. Biotechnol., 19: 159-164.