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Original Research Article

Effect of *Costus afer* on Carbohydrates Tolerance Tests and Glucose Uptake

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Abstract: Diabetes is commonly characterized by hyperglycemia related to different mechanisms. Many plants have been reported to possess antidiabetic actions in lowering blood sugar concentration and one of such is *Costus afer*. The present study hypothesis is that methanolic extract of *C. afer* (leaf, stem, and rhizome) control hyperglycemia by inhibiting glucose uptake. Glucose uptake inhibition was carried out by incubating glucose with plant extract of varying dosages in yeast cells. The carbohydrate tolerance tests characterized by starch and maltose tolerance tests were carried out by loading two sets of experimental animals with respective carbohydrate and varying dosages of *C. afer* extract. In both studies metformin was used as the reference drug. *C. afer* extracts significantly inhibited the uptake of glucose by yeast cells. The percentage inhibition was below 50 % for all extracts with a dose dependent effect. The methanolic leaf extract of *C. afer* had the highest effect followed by stem and rhizome. The inhibition potential of *C. afer* leaf extract was comparable to that of metformin the reference drug. Similar results were obtained in the carbohydrate tolerance test. Groups of rats treated with plant extracts had lower blood glucose level compared to the groups that treated with carbohydrates only. Metformin was significantly better than plant extracts in the lowering of blood sugar. The result obtained is a proof of concept that *C. afer* possess antidiabetic properties though more need to be done to effectively confirm its use as an antidiabetic agent.
Keywords: *Costus afer*, metformin.

INTRODUCTION

In individuals with type 2 diabetes, nutrient intake related first-phase insulin response is severely diminished or absent resulting in persistently elevated postprandial glucose (PPG) throughout most of the day[1].This may be due to the delayed peak insulin levels which are insufficient to control PPG excursions adequately[2] .Postprandial hyperglycemia is a major risk factor for micro- and macro vascular complications associated with diabetes[3,4] and so controlling postprandial plasma glucose level is critical as a measure to the early treatment of diabetes mellitus and in reducing chronic vascular complications[5]. Hence, decreasing postprandial hyperglycemia is a therapeutic approach for management of type 2 diabetes (T2D). T2D is characterized by a reduced sensitivity to insulin signaling and a reduced efficiency of glucose transport, primarily in adipocytes and muscle cells, leading to hyperglycemia and hyperinsulinemia.[6] Glucose uptake and storage in peripheral tissues such as skeletal muscles and adipose tissue is a major regulatory process in the homeostatic control of blood glucose levels [7].

Skeletal muscle, by virtue of its large contribution to body mass, represents the major site of insulin-mediated glucose disposal. However, both tissues contribute toward the lowering of blood glucose[8]. Glucose uptake is mediated through the translocation of the Glut4 receptor from the interior to the cell surface which is stimulated by the insulin signaling pathway initiated by activation of the insulin receptor. Any defect in this pathway triggers the development of hyperglycemia in type II diabetes. Thus, measurement of glucose uptake into peripheral tissues is an important mechanism to assess insulin sensitivity.

New anti-diabetic compounds which function by this mechanism but devoid of side effect are therefore desirable. *C. afer* , belongs to the

Zingiberaceae family and it is commonly known as bush sugar cane or monkey sugar cane[9] commonly found in moist and shady forest of West and Tropical Africa [10]. In an earlier study we demonstrated the antioxidant properties of *C. afer* and its potentials for inhibiting the activity of carbohydrates metabolizing enzymes[11]. Hence the present study evaluates the methanolic extract of each part of *Costus afer*(Leaf, Stem and Rhizome) against glucose uptake by yeast cells and carbohydrates tolerance tests on postprandial glucose excursion associated with polysaccharide and disaccharide challenge in normal rats.

MATERIALS AND METHODS

Plant material

Fresh *Costus afer* (leaf, stem and rhizome) were collected from their natural habitat in Yaoundé-Cameroon and Voucher specimens (HNC 11708) were deposited at the Yaoundé National herbarium. Fresh samples were cleaned with tap water, chopped into small pieces, and air-dried at room temperature to constant weight. The dried samples were then pulverized into fine powder. The methanolic extracts from different parts of *C. afer* were obtained from the powder as previously described [11].

Animals

Male Wistar rats produced in the pharmacology laboratory of Institute of Medical Research and Medicinal Plants Studies (IMPM) weighing 160-220g were acclimatized at the temperature of 23 ± 2°C with controlled humidity conditions (50-55%) at 12 h light and dark cycle. The rats were kept in polypropylene cages and were fed ad libitum. .

Glucose uptake in Yeast cells

Yeast cells were prepared according to the method earlier described by Gupta *et al.;* [12] Briefly, commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids became clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (10–50µg/ml) were added to 1 mL of glucose solution (5, 10 and 20mM) and incubated for 10 min at 37 °C. Reaction was initiated by the addition of 100 µl of yeast suspension, vortex and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (3000rpm, 5 min) and glucose was estimated in the supernatant. Metformin was used as standard (reference) drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

% inhibition of glucose uptake = ((Abs sample – Abs control)/Abs control) * 100.

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

Carbohydrates Tolerance Tests in Normal rats

Oral Starch Tolerance Test (OSTT)

Thirty rats were divided into six groups of five rats (n = 5) each and treated as follows Group I (normal control rats, distilled water), Group II (diabetic control, loaded with starch and distilled water), Group III (metformin, reference drug), Group IV (250 mg/Kg), Group V (500 mg/Kg), and Group VI (1000 mg/Kg). This distribution was applied to all the extracts of *C. afer* tested. After an overnight fast (12 h), the blood glucose of all groups was taken and considered as time zero (0) before the administration of plant extract. Thirty minutes after administration of plant extract (or distilled water or Metformin), all rats were administered starch (3 g/kg body mass) orally. Postprandial blood glucose levels were then measured at 30, 60, 90, 120 and 180 min after oral administration of starch using Glucometer Accu-check (Bayer Diagnostics, Germany). Postprandial blood glucose (PBG) curves were plotted and the area under the curve (AUC) calculated [13].

Oral Maltose Tolerance Test (OMTT)

The procedure for maltose tolerance test was similar to that of starch tolerance tests except that maltose (5 g/kg body mass) instead of starch was orally administrated to all groups of rats [13].

AUC determination was calculated as follows:

AUC (mM/ (h) = $((BG_0+BG_{30})/2)*0.5$ + $((BG_{30}+BG_{60})/2)*0.5$ + $((BG_{60}+BG_{90})/2)*0.5$ + $((BG_{90}+BG_{120})/2)*0.5$ + $((BG_{120}+BG_{180})/2)*1$). BG: Blood Glucose.

STATISTICAL ANALYSIS

The values are expressed as mean ± S.E.M. Statistical difference in PBG and AUC between control and treatment groups was determined using Statistical Package for Social Sciences (SPSS) 20.0 software one-way analysis of variance (ANOVA) followed by Tukey's test for post hoc analysis. $P< 0.05$ was considered as significant.

RESULTS

In vitro Glucose uptake in Yeast cells

The effect of plant extract on glucose uptake across yeast cell membrane system is presented in Table 1A-C. All three plants extracts show inhibition of glucose uptake at all three glucose concentrations studied. The % inhibition of glucose uptake in the yeast cell was linear with increasing glucose concentration of 5, 10 and 20 mM, respectively. The methanol leaf extract of *Costus afer* exhibited the highest inhibition activity in the three glucose concentrations (Table 1A, 1B, 1C). Results also indicated that *Costus afer* methanolic extracts had comparative effect to metformin in inhibiting glucose uptake in yeast cells while the rhizome extract had the least inhibitory effect.

Table 1A:% inhibition of Glucose uptake in 20mM glucose concentrations

Glucose 20 mM				
Extract (µg/ml)	**Metformin (%Inh)**	**MeOH Leaf (%Inh)**	**MeOH Stem (%Inh)**	**MeOH Rhizome (%Inh)**
50	33.66±0.28	34.44±0.12	32.68±0.23	31.41±0.92
40	28.87±0.61	23.12±1.25	31.27±0.69	29.35±0.34
30	24.58±2.09	12.52±0.74	29.49±3.4	26.84±0.17
20	16.43±0.40	10.91±0.48	28.17±1.87	18.97±0.24
10	5.66±0.36	5.34±1.19	25.93±5.00	10.68±0.86

Table 1B: % inhibition of Glucose uptake in 10mM glucose concentrations

Glucose 10 mM				
Extract (µg/ml)	**Metformin (%Inh)**	**MeOH Leaf (%Inh)**	**MeOH Stem (%Inh)**	**MeOH Rhizome (%Inh)**
50	28.08±0.46	29.07±0.82	25.18±0.46	17.89±3.50
40	17.81±0.63	26.31±0.54	22.03±0.50	11.34±0.87
30	14.87±1.17	21.52±3.68	19.86±1.04	4.29±1.13
20	7.94±0.62	19.51±3.19	14.44±1.69	0.67±0.16
10	3.91±0.81	11.35±0.51	6.09±4.37	

Table 1C: % inhibition of Glucose uptake in 5 mM glucose concentrations

Glucose 5mM				
Extract (µg/ml)	**Metformin (%Inh)**	**MeOH Leaf (%Inh)**	**MeOH Stem (%Inh)**	**MeOH Rhizome (%Inh)**
50	25.37±0.74	26.69±1.41	19.93±0.95	14.77±0.93
40	20.06±2.46	19.86±1.57	16.11±1.04	8.95±1.86
30	14.52±1.33	7.67±1.58	13.56±0.67	
20	1.47±0.97	1.15±0.57	8.59±1.29	
10			3.04±0.84	

Results are presented as mean ± SD for percentage inhibition. Samples were analyzed in triplicate

Carbohydrate tolerance tests in normal rats

The effect of the methanol extract of different parts of *C. afer* on post-prandial hyperglycemia (PPHG) was studied in normal rats by means of oral starch and maltose tolerance tests.

Effect of methanolic extracts on PPHG in healthy rats loaded with starch (Oral starch tolerance test)

In the oral starch test, the postprandial blood glucose variation was measured after loading experimental animals with starch (Fig 1 A&B). Thirty minutes after the administration of starch the PPHG increased in all the groups except the 500mg/ml extract and metformin treated groups that experience a drop (Fig 1A). The highest increase in PPHG was observed in the negative or diabetic control group at 60 minutes of the administration of starch meanwhile the rest of the groups experience a drop in their PPHG. This increase in PPHG is accountable for the large AUC observed for the negative control animals compared to the group of animals treated with metformin and C afer leaf extract (Fig 1B).

Figure 2 A&B presents the effect of C afer stem extract on postprandial blood glucose of experimental animals loaded with starch while Fig 3 A&B presents the effect of C afer rhizome extract on postprandial blood glucose of experimental animals loaded with starch. The results obtained for both stem (Fig 2 A&B) and rhizome (Fig 3 A&B) methanolic extracts were very similar with those of the methanolic leaf extract. The negative controls had the highest peaks (Fig 2A & 3A) and the largest area under the curve (Fig 2B & 3B) which were significantly different ($P<0.05$) from the normal control rats. The increase in postprandial glucose concentration was inhibited in the groups administered the C afer extracts towards normal. However, metformin showed the lowest postprandial glucose concentration compared to the rest of the group ($P<0.01$) and better than all the extracts of C afer. With exception of the negative control that the glucose concentration remains high, the rest of the glucose concentration that increased at 30 minutes dropt towards normal at 60 minutes defining the recovery or treatment phase.

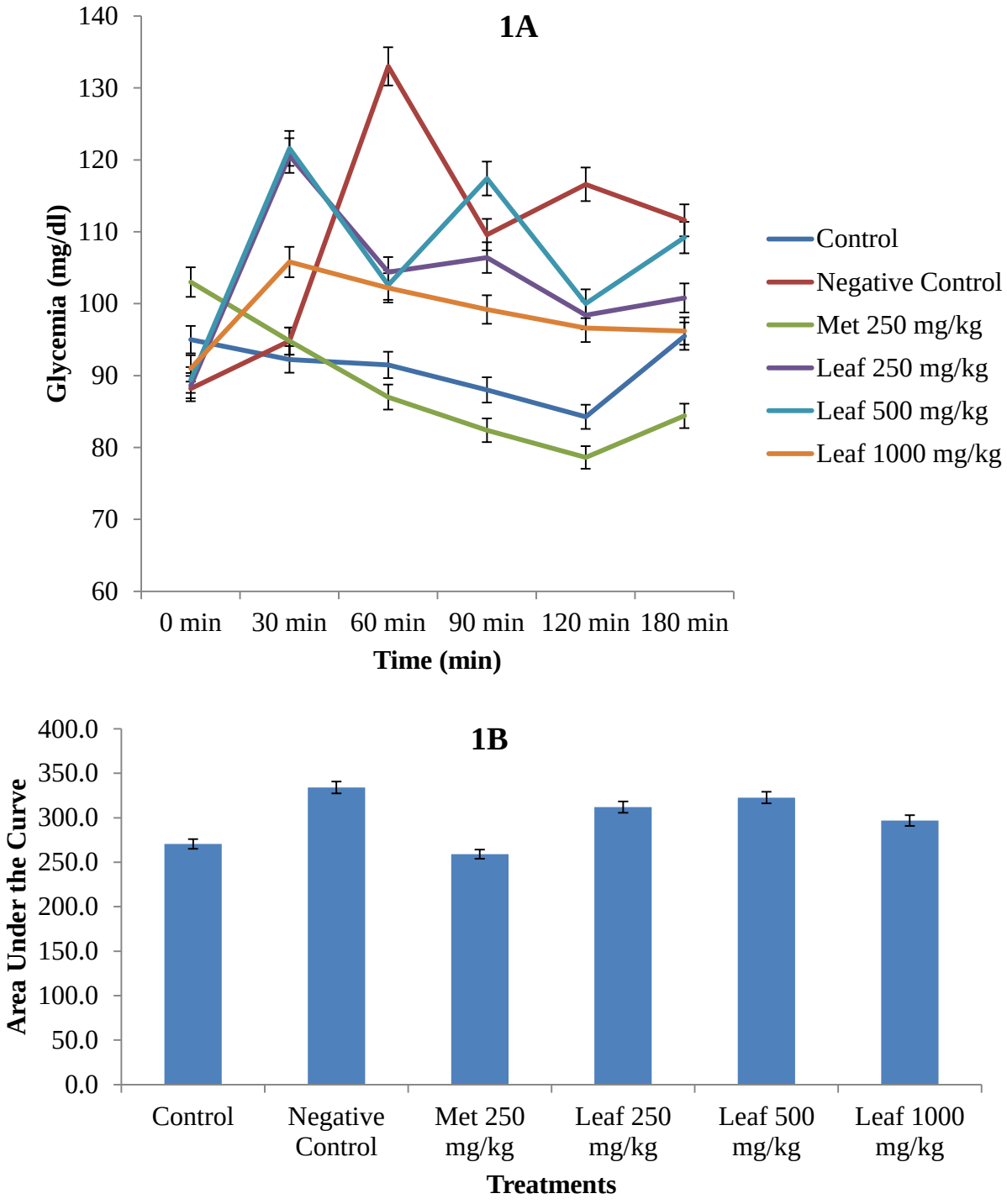
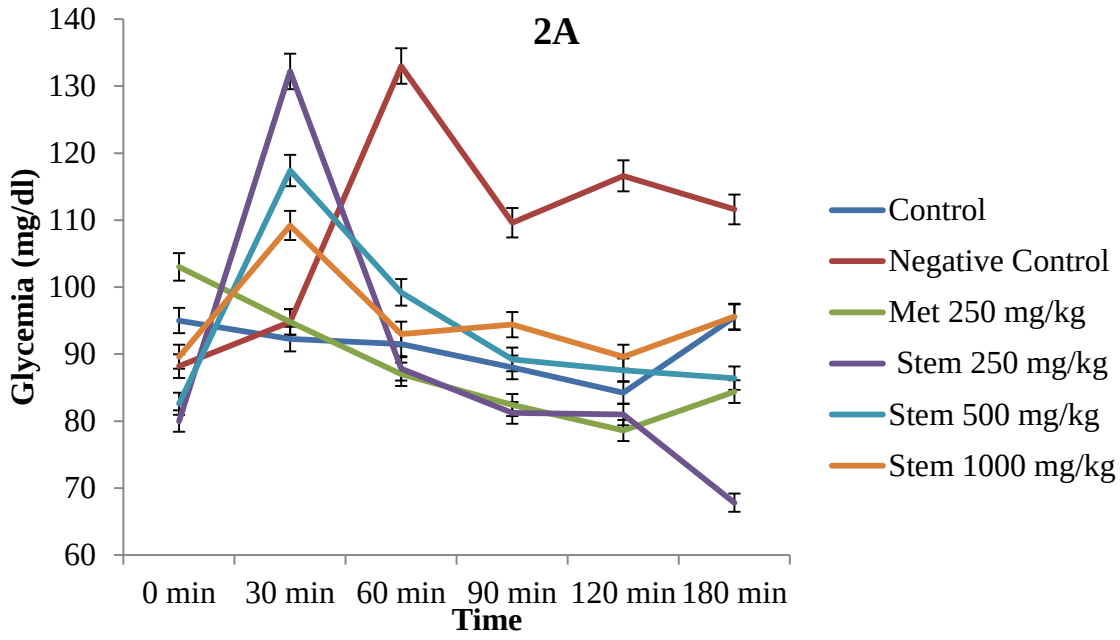


Fig 1: Effect of methanolic leaf extract on PPHG of experimental rats loaded with starch: (A) = Blood glucose concentration with respect to time after loading experimental animals with starch. (B) = Area under the respective curves of A. Results is presented as mean ± standard deviation of PPHG analysis done in triplicates. Control: received just the vehicle (distilled water); Negative Control: received starch and water; Met 250: received standard (Metformin) and starch.



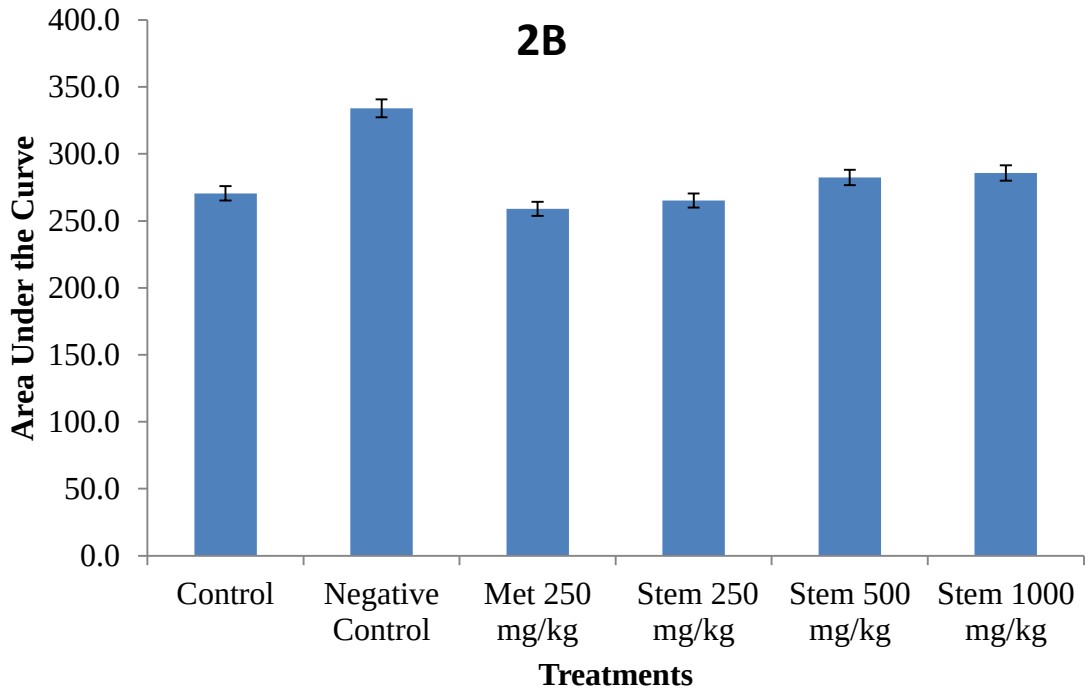


Fig 2: Effect of methanolic stem extract on PPHG of experimental rats loaded with starch: (A) = Blood glucose concentration with respect to time after loading experimental animals with starch. (B) = Area under the respective curves of A. Results is presented as mean ± standard deviation of PPHG analysis done in triplicates. Control: received just the vehicle (distilled water); Negative Control: received starch and water; Met 250: received standard (Metformin) and starch.

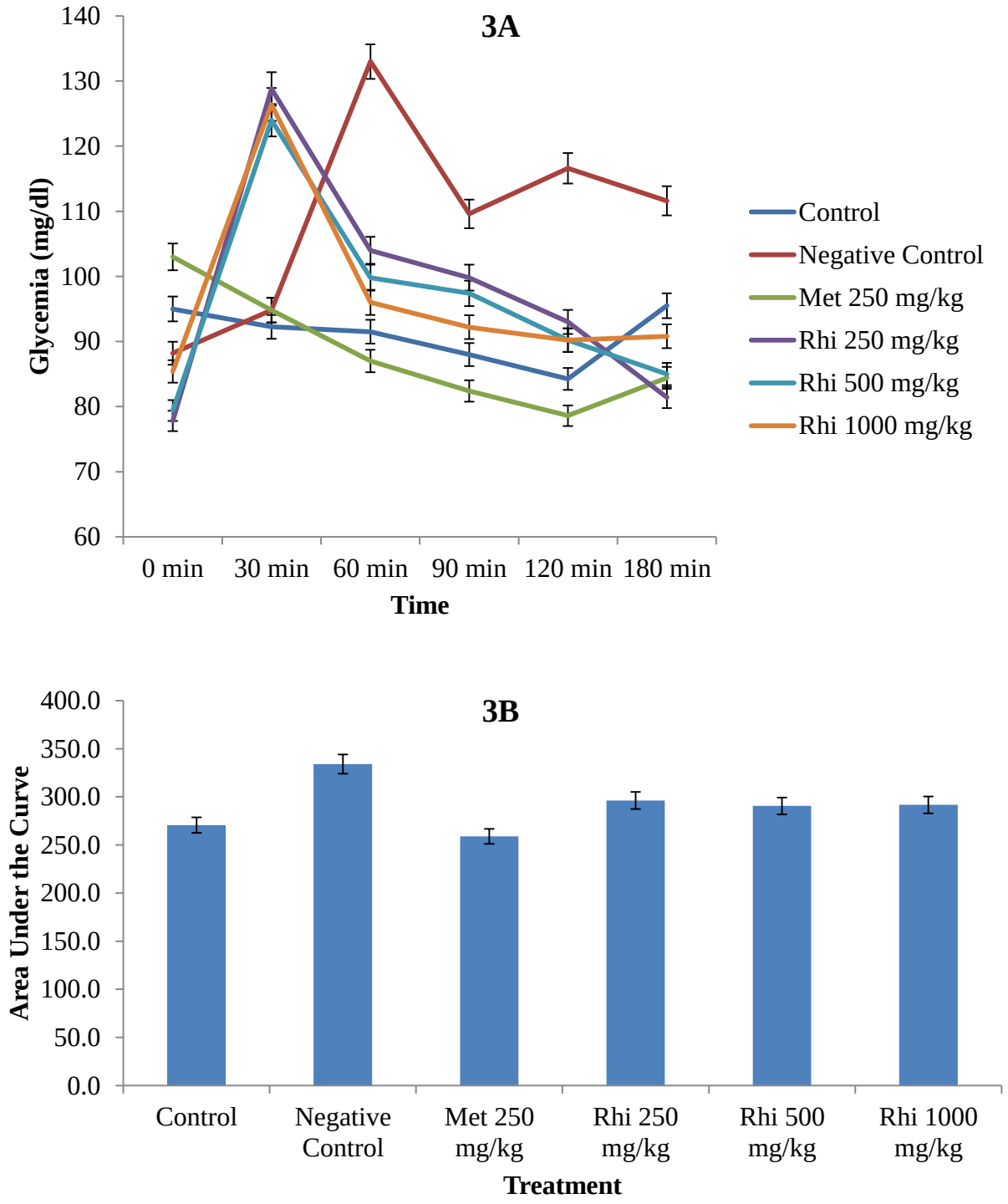
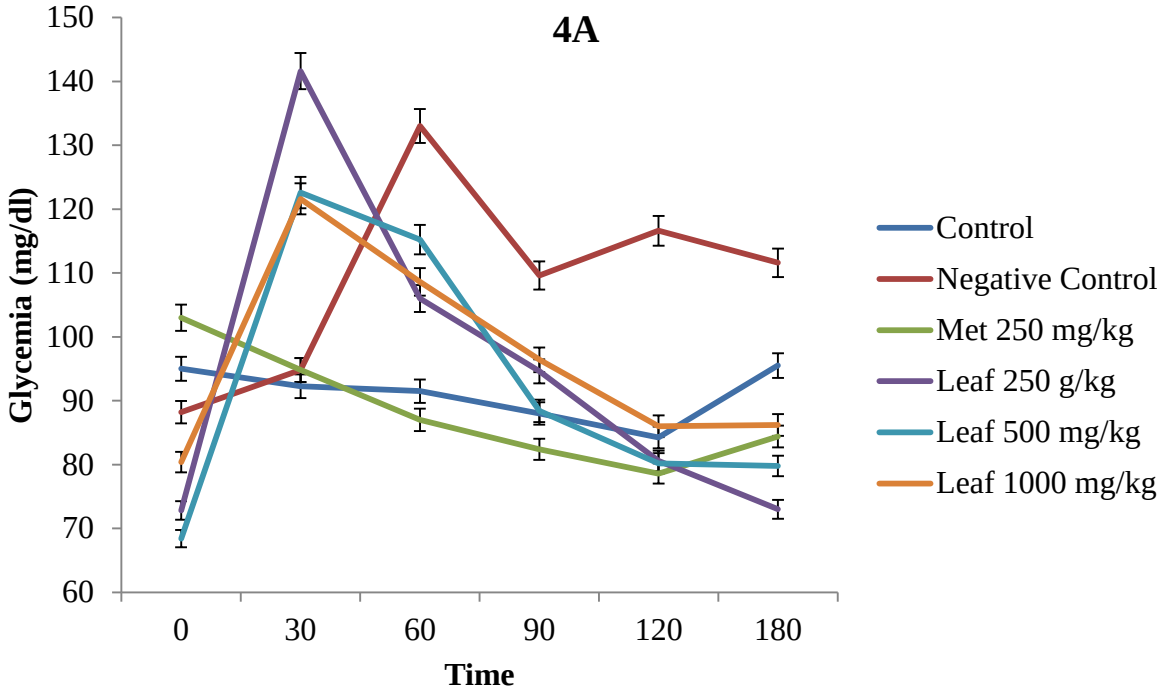


Fig 3: Effect of methanolic rhizome extract on PPHG of experimental rats loaded with starch: (A) = Blood glucose concentration with respect to time after loading experimental animals with starch. (B) = Area under the respective curves of A. Results are presented as mean ± standard deviation of PPHG analysis done in triplicates. Control: received just the vehicle (distilled water); Negative Control: received starch and water; Met 250: received standard (Metformin) and starch.

Effect of methanolic extracts on PPHG in healthy rats loaded with maltose

Oral maltose Tolerance Test: Postprandial blood glucose variations was measured after loading maltose to the normal rats with and without the co-administration of *C. afer* extract are presented in Figure 4, 5 & 6. In the negative control groups, blood glucose level increased at 30 minutes after the maltose load. In the group that received *C. afer* extracts (leaf, Fig. 4 A&B; stem, Fig. 5 A &B; rhizome, Fig. 6 A &B) alongside maltose, the 30 minutes post-load glucose level increased in all plant parts (Figure 4, 5 and 6).

This increasing of blood glucose in OMTT more than in OSTT indicates the speed conversion of maltose in 2 molecules of glucose compared to starch that is a polysaccharide. Compared to negative control, the whole glycemic response is increased after 60 minutes on *C. afer* treatment with blood glucose dropping towards normal concentrations.



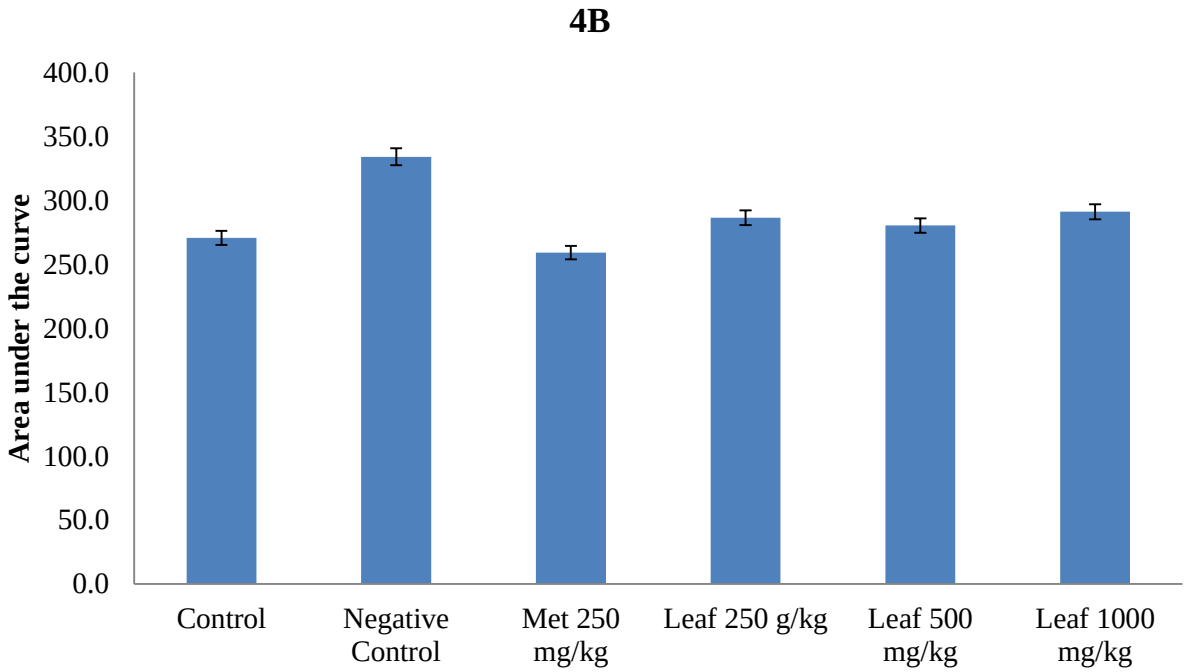


Fig 4: Effect of methanolic leaf extract on PPHG of experimental rats loaded with maltose: (A) = Blood glucose concentration with respect to time after loading experimental animals with starch. (B) = Area under the respective curves of A. Results is presented as mean ± standard deviation of PPHG analysis done in triplicates. Control: received just the vehicle (distilled water); Negative Control: received starch and water; Met 250: received standard drug (Metformin) and maltose.

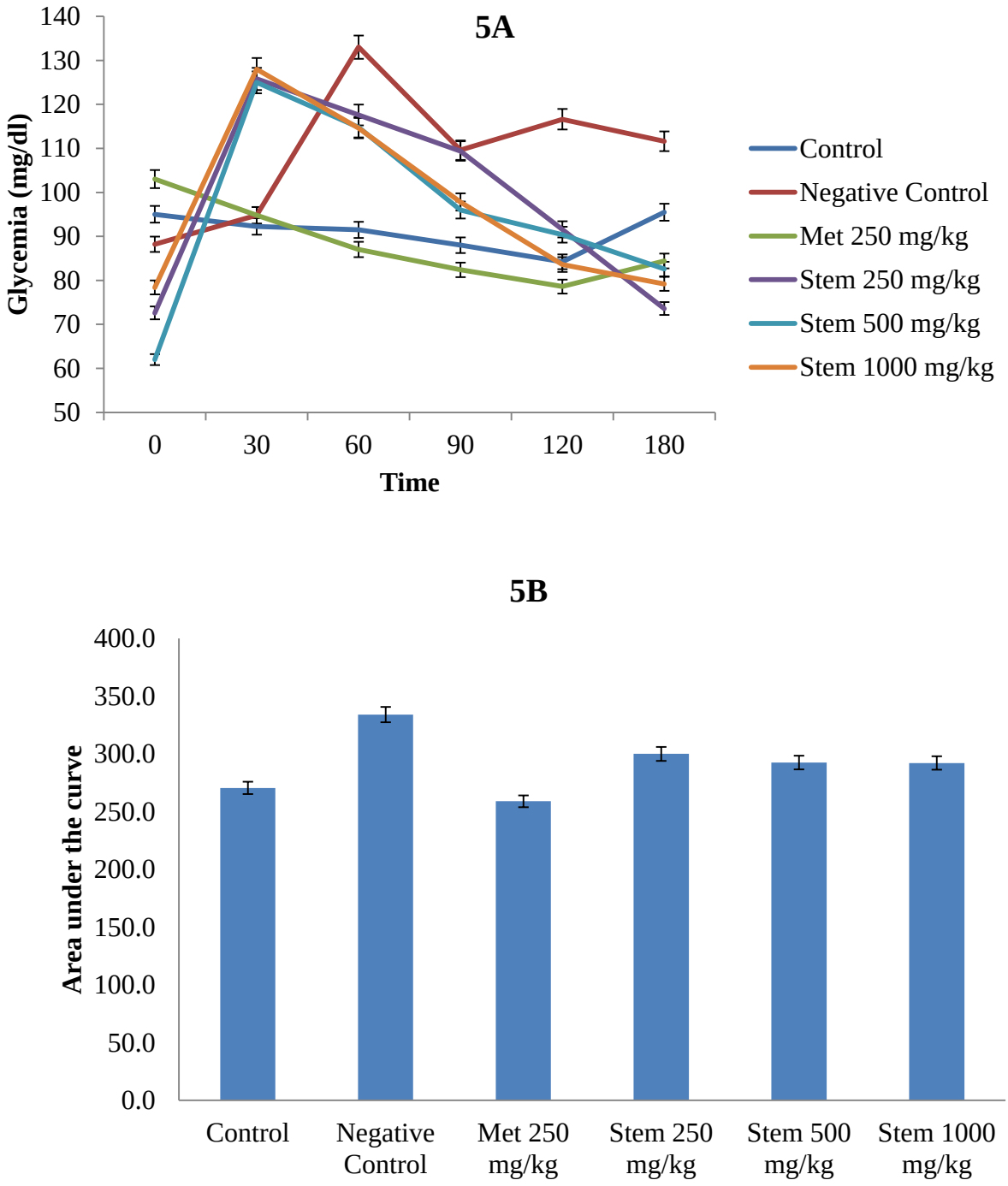


Fig 5: Effect of methanolic stem extract on PPHG of experimental rats loaded with maltose: (A) = Blood glucose concentration with respect to time after loading experimental animals with starch. (B) = Area under the respective curves of A. Results is presented as mean ± standard deviation of PPHG analysis done in triplicates. Control: received just the vehicle (distilled water); Negative Control: received starch and water; Met 250: received standard (Metformin) and maltose.

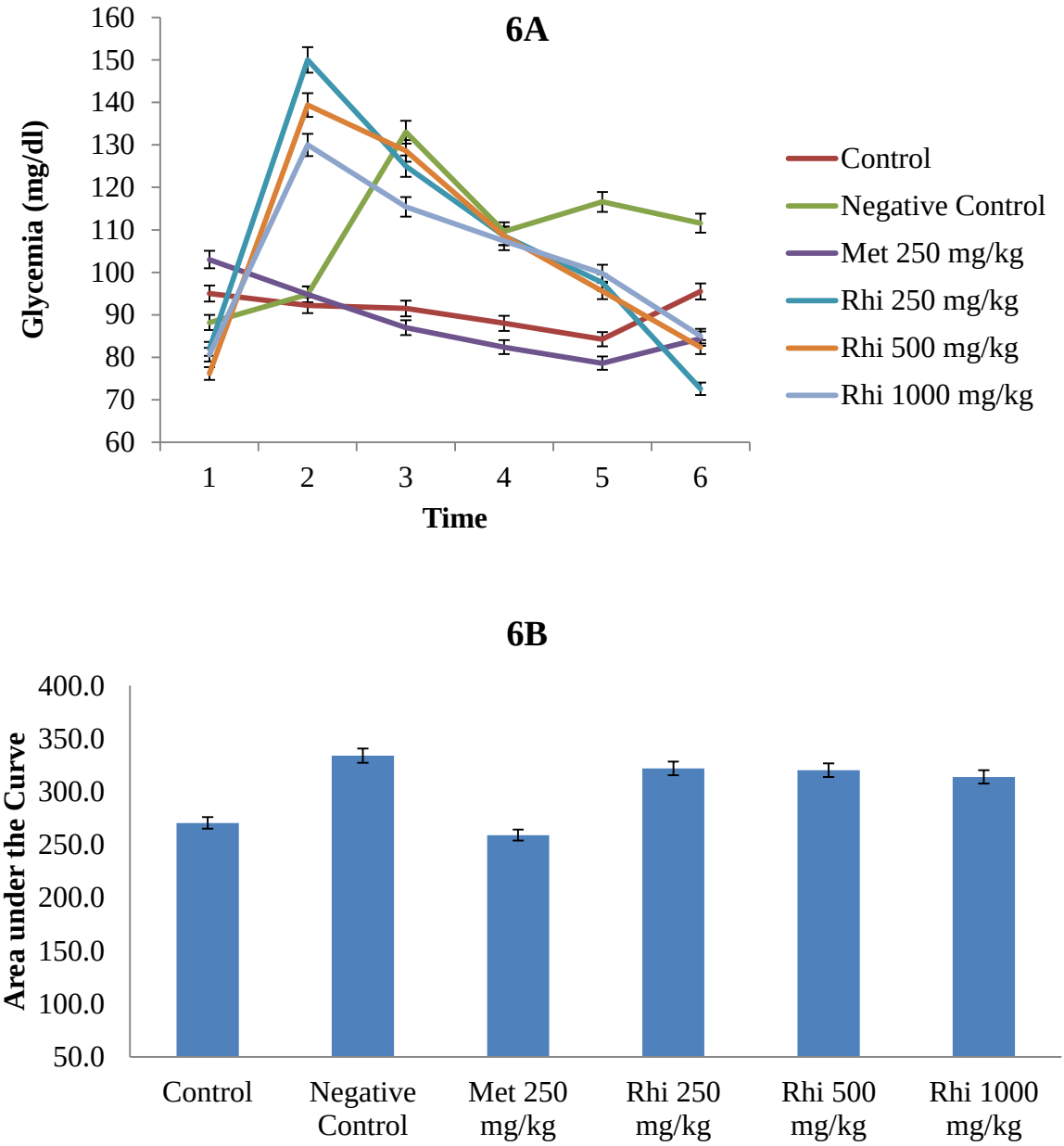


Fig 6: Effect of methanolic rhizome extract on PPHG of experimental rats loaded with maltose: (A) = Blood glucose concentration with respect to time after loading experimental animals with starch. (B) = Area under the respective curves of A. Results is presented as mean ± standard deviation of PPHG analysis done in triplicates. Control: received just the vehicle (distilled water); Negative Control: received starch and water; Met 250: received standard (Metformin) and maltose.

DISCUSSION

Diabetic individuals are at an increased risk of developing micro vascular complications (retinopathy, nephropathy, and neuropathy) and cardiovascular disease (CVD). Abnormalities in insulin and glucagon secretion, hepatic glucose uptake, suppression of hepatic glucose production, and peripheral glucose uptake contribute to higher and more prolonged postprandial glycemic (PPG) excursions than in non-diabetic individuals [2].The present study evaluates the antidiabetic properties of C afer as characterized in glucose uptake in yeast cells and oral carbohydrate tolerance test. The biguanide metformin which is currently used for lowering blood glucose in diabetic patients was used as the reference drug. The cell membrane is very important for the movement of materials in and out of the cell. Some movements occur only by simple diffusion in respond to a concentration gradient in which case the rate is controlled by the permeability of the membrane and the magnitude of the concentration gradient. Some other molecules move through the cell membrane by active transport with or facilitated transport by specialized proteins. In recent times the study of glucose uptake has been receiving

renewed attention in many laboratories with yeast cell as the model of study [14-16]. Earlier studies on the uptake of nonmetabolizable sugars [17-23] and certain metabolizable glycosides [24] have suggested that the transport across the yeast cell membrane is mediated by stereospecific membrane carriers. The carrier hypothesis explains that the cell membrane contains stereospecific carriers which diffuse in both directions and at the same rate, whether combined or uncombined. The process is believed to involve three consecutive reactions: combination between the substrate and carrier to form a carrier-substrate complex, diffusion of the carrier-substrate complex across the cell membrane, and dissociation of the carrier-substrate complex into free substrate and carrier [25]. In the present study, the C afer methanolic extracts were first incubated with the glucose solution expecting the formation of the substrate-inhibition complex. This prevents the carrier (transporter) from forming the substrate-carrier complex with the result of inhibition of glucose uptake observed in this study.

The yeast glucose transporters are highly conserved and transport glucose in a similar mechanism as human SGLT1 [26] mainly expressed in the small intestine for glucose absorption and to some extent in the kidney where it contributes to glucose reabsorption [27-29]. Hence inhibition of this protein (SGLT1) will lead to malabsorption of glucose and may be useful therapeutic approach for the management of type 2 diabetes. This is because it inhibits absorption of glucose from the intestinal wall and inhibits the reabsorption of glucose in the kidney with the final results of much of the glucose being excreted. SGLT2 co-transporters are responsible for reabsorption of most (90 %) of the glucose filtered by the kidneys. The pharmacological inhibition of SGLT2 co-transporters reduces hyper glycaemia by decreasing renal glucose threshold and thereby increasing urinary glucose excretion. Increased renal glucose elimination also assists weight loss and could help to reduce blood pressure [30]. We here infer that, since the yeast glucose transporters are similar in function and operate same mechanism with the human intestinal glucose transporter (SGLT1), it is possible that C afer extract binds glucose and inhibit its binding with SGLT1 or forms a complex with SGL1 that cannot be transported across the membrane.

Oral carbohydrate tolerance test was carried out to further substantiate the antidiabetic activity of C afer extracts. The acute antihyperglycemic activity of MeOH extract of all part of *C. afer* (leaf, stem and rhizome) was evaluated in normoglycemic rats during Oral carbohydrates Tolerance Tests (OCTT). OCTT data over 3 h indicates that leaf and stem extracts reduced plasma glucose concentration in the oral carbohydrate loaded rats. The other species of Costus (Costuspictus) have also been reported to have similar effects with methanolic extract [31]. These extracts at a dose of 1000 mg/kg significantly lowered AUC, when compared with negative control group. The decline is more pronounced with metformin (standard) but similarity to the effects of extracts. These findings suggested that MeOH leaf and Stem extracts of *C. afer* could decrease the postprandial blood glucose level by inhibiting the activity of a-amylase and a-glucosidase as earlier reported [11] which are important enzymes in the digestion of the complex carbohydrates into absorbable monosaccharides in the food [32]. Another hypothesis could be that *C. afer* extracts bind the intestinal glucose and prevents it from being absorbed or transported as discussed above.

The significant reduction of AUC values caused by metformin and extracts in OCTT indicates hypoglycemic effect. Metformin enhances glucose utilization by peripheral tissues and also a delay in the absorption of glucose [33]. It stimulates the hepatic enzyme AMP-activated protein kinase [5]. If insulin secretion is induced before glucose loading, plasma glucose concentration will not rise significantly above normal range 2h post prandial. *C. afer* extracts could possibly be exerting its hypoglycemic effect by mechanisms similar to that of metformin.

It is worth concluding that C afer regulate postprandial blood glucose by inhibiting intestinal glucose uptake as shown with yeast cell in a dose related manner.

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