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ORIGINAL ARTICLE

In vitro Hypoglycemic Effects of Molokhia Leaves (*Corchorus olitorius* L.)

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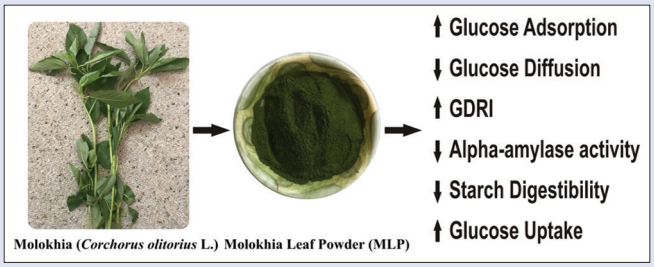
ABSTRACT

Background: *Corchorus olitorius* L., commonly known as molokhia in Arabic, is a leafy vegetable containing large amounts of mucilaginous polysaccharides and reported to exhibit antidiabetic diabetic activity in experimental diabetes. Although antidiabetic effect of molokhia is attributed to the presence of phenolic and non-phenolic compounds, the precise mechanism of action is not explored. **Materials and Methods:** The present study investigated the ability of molokhia leaves powder (MLP) to modulate starch digestion and glucose diffusion *in vitro*. **Results:** MLP at 2% and 4% levels bound significantly higher amount of glucose than wheat bran (2%) and also reduced diffusion of glucose through dialysis membrane as reflected by significantly higher glucose dialysis retardation index. In starch–α-amylase system, MLP (4%) showed complete inhibition of starch digestion and glucose diffusion similar to that of acarbose till 120 min. Further, glucose diffusion was retarded to an extent of 83.7% and 63.5% by MLP (4%) at 180 and 240 min, respectively. On the other hand, MLP (2%) retarded glucose diffusion to an extent of 96%, 65%, and 51% at 120, 180, and 240 min respectively. Furthermore, molokhia leaf extract significantly enhanced uptake of glucose by rat hemidiaphragm *in vitro*. **Conclusion:** These findings conclusively demonstrate that the antidiabetic effect of molokhia leaves is mediated through delaying starch digestion and physical adsorption of liberated glucose limiting its diffusion across intestinal lumen and enhancing glucose uptake in peripheral tissues.

Key words: *Corchorus olitorius*, *in vitro* hypoglycemic, molokhia, starch digestion, α-amylase

SUMMARY

- Molokhia is known to exhibit antidiabetic effect which is attributed to phenolic compounds and polysaccharides. This study explored the mechanism of antidiabetic action of molokhia leaves using suitable *in vitro* techniques. The findings demonstrated that molokhia leaves exert their antidiabetic effect by delaying starch digestion via higher glucose adsorption thereby limiting its diffusion across intestinal lumen and enhancing glucose uptake in peripheral tissues.



Abbreviations used: MLP: Molokhia leaf powder; MLE: Molokhia leaf extract; GDRI: Glucose dialysis retardation index.

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INTRODUCTION

Diabetes mellitus is a multietiological and heterogeneous disorder of metabolism characterized by raised serum glucose levels associated with a wide range of health complications and economic burden.[1] Although several therapeutics are available for managing diabetes, exploring dietary polysaccharides for their antidiabetic potential has gained much attention by researchers of late. A number of polysaccharides isolated from dietary sources including oats, mushrooms, beans, pumpkin, and cucumber have been reported to possess significant hypoglycemic effects associated with reduced diabetic complications by reducing oxidative stress and improving insulin sensitivity.[2-5] A water-soluble polysaccharide obtained from *Lycium barbarum* fruits contained mannose, rhamnose, and glucose as repeating units exhibited dose-dependent inhibition of glucose absorption in experimental animals.[6] Another polysaccharide containing rhamnose, arabinose, mannose, and galactose as repeating units is reported to exhibit strong antidiabetic effect in experimentally-induced diabetic rats,[5] while another water-soluble polysaccharide containing L-rhamnose, D-xylose, L-arabinose, D-glucose, D-mannose, and D-galactose also exhibited potent hypoglycemic effect in alloxan-induced diabetic rats.[7] These observations prompted researchers to explore dietary sources of complex polysaccharides for their utilization as therapeutics in diabetes.[1]

Corchorus olitorius L., commonly known as molokhia in Arabic, is one such leafy vegetable containing mucilaginous polysaccharides similar to okra. Among various soluble polysaccharides that are abundantly present in molokhia leaves, an acidic water-soluble polysaccharide containing uronic acid, rhamnose, glucose, galacturonic acid, and glucuronic acid has been isolated.[8] Of various biological effects, molokhia leaves and seeds have been reported to exhibit potent antidiabetic activity in experimental diabetes.[9-18] The reported antidiabetic effects of molokhia were attributed to the occurrence of flavonoids, alkaloids, terpenoids,

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steroids, and complex viscous carbohydrates.[18] Dietary components can exert hypoglycemic effect through delaying starch digestion by inhibiting carbohydrate enzymes, inhibiting glucose diffusion by entrapping liberated glucose in the gastrointestinal (GI) tract, and increasing glucose uptake in target tissues.[19,20] Aqueous extract of molokhia leaves has been reported exhibiting strong inhibitory effects against carbohydrate hydrolyzing enzymes, viz., α-amylase and α-glucosidase. It was hypothesized that the enzyme inhibitory effect is due to the occurrence of phenolic and non-phenolic components, possibly polysaccharides present in molokhia leaves.[21] Similar findings are also reported for some medicinal plants used in Latin America having potential α-amylase inhibitory activity.[22]

The aforementioned studies do not illustrate the mechanism of action for the antidiabetic effect of molokhia. Thus, the present study investigates the possible mechanism of hypoglycemic action of molokhia leaves using suitable *in vitro* techniques.

MATERIALS AND METHODS

Materials

Molokhia leaves and wheat bran (WB) were procured from a departmental store, while acarbose and regular insulin (0.4 U/mL) were purchased form a local pharmacy. Dialysis bag (12 KD cutoff), glucose oxidase peroxidase (GOD-POD) assay kit, and α-amylase were procured from Sigma Aldrich, Saudi Arabia. Only laboratory-grade reagents and chemicals were utilized in the study.

Processing of the sample

Molokhia leaves were separated from stems and washed in tap water. The leaves were spread on the trays and dried in shade. The dried leaves were powdered to pass through 60 mesh sieve (BS) using a compact cyclonic laboratory blender to yield molokhia leaf powder (MLP), which was stored in an air-tight container in a refrigerator for subsequent use.

Measurement of glucose adsorption capacity of molokhia leaf powder

MLP's glucose adsorption capacity was measured using the *in vitro* method defined by Ahmed and Urooj.[19] In 50 mL centrifuged tubes, MLP (500 and 1000 mg) was added to 25 mL of glucose solution of increasing concentrations (5, 10, 20, 50, and 100 mM) and vortexed for 10 s. After that, the tubes were incubated in an automated shaking water bath set at 37°C. The speed was set at 90 cycles/min. The tubes were centrifuged at 4000 g for 20 min after 6 h of incubation to assess the glucose content in the supernatant using GOD-POD assay. The amount of glucose bound by MLP was measured using the formula below and expressed in millimoles (mM). As a control, 2% WB (500 mg) was used.

$$\text{Glucose Bound} = \frac{\text{Glucose concentration of original solution - glucose concentration after 6h}}{\text{Weight of the sample}} \times \text{Volume of solution}$$

Measurement of the effect of molokhia leaf powder diffusion of glucose through dialysis membrane

The influence of MLP on retardation of glucose diffusion across dialysis membrane (12 KD) was investigated as reported earlier by Ahmed and Urooj.[19] MLP at two levels (500 and 1000 mg) were added into a dialysis bag along with 25 mL of glucose solution (20 mM). The dialysis bag was sealed and placed into 250 mL tall form beakers containing 200 mL double distilled water. The beakers were incubated in an automated shaking water bath set at 37°C. The amount of glucose diffused out into the dialysate after 60, 120, 180, and 240 min of incubation was measured by GOD-POD assay kit. As a control, 50 mg of acarbose (0.2%) was used. The following formula was used to measure the glucose dialysis retardation index (GDRI).

$$\text{GDRI} = 100 - \frac{\text{Glucose content with addition of sample}}{\text{Glucose content of control}} \times 100$$

Molokhia leaf powders effect on amylolysis kinetics

MLP's effect on amylolysis kinetics was studied using an *in vitro* method described by Ahmed and Urooj.[19] Forty grams of potato starch was taken in a beaker containing 0.9 L of phosphate buffer (0.05 M; pH 6.5). A 2-inch-long magnetic bead was added into the beaker and heated to 65°C on a magnetic stirring hot plate. After 30 min, the solution was transferred to 1-L volumetric flask, and the volume was made up to the make to obtain starch solution of 4% strength.

Amylolysis kinetic assay was carried out by adding MLP at two levels (500 and 1000 mg) into a dialysis bag along with 25 mL of starch solution and 100 mg of α-amylase. The dialysis bag was sealed and placed into 250 mL tall form beakers containing 200 mL double distilled water. The beakers were incubated in an automated shaking water bath set at 37°C. The amount of glucose diffused out into the dialysate after 60, 120, 180, and 240 min of incubation was measured by GOD-POD assay kit. A test without MLP as used as a control and a test with 50 mg of acarbose (0.2%) was used as a reference. The following formula was used to measure the GDRI.

$$\text{GDRI} = 100 - \frac{\text{Glucose content with addition of sample}}{\text{Glucose content of control}} \times 100$$

Effect of molokhia leaf extract on rat hemidiaphragm glucose uptake

The effect of molokhia leaf extract (MLE) on glucose taken up by the rat hemidiaphragms was investigated *in vitro*.[19] MLE was obtained by extracting MLP with distilled water at 70°C for 24 h on a mechanical shaker. After that, the extract was filtered and freeze-dried. The dried extract was redissolved in distilled water to prepare working stock solution of 10 mg/mL concentration. Diaphragms harvested from overnight-fasted rats were divided into two halves and placed in stoppered tubes containing 2 mL Tyrode solution. The tubes were divided into four groups ($n = 6$).

- Group 1: Tyrode solution (2 mL) +2 mL distilled water
- Group 2: Tyrode solution (2 mL) +0.5 mL insulin + 1.5 mL distilled water
- Group 3 – Tyrode solution (2 mL) +1.5 mL MLE + 1.5 mL distilled water
- Group 4 – Tyrode solution (2 mL) +0.5 mL insulin + 1.5 mL MLE.

The tubes with their corks open were incubated at 37°C in 100% oxygen atmosphere chamber for 30 min while being shaken at 140 cycles per minute. The differential between the initial and final glucose concentrations of the reaction mixture was used to quantify glucose uptake, which was expressed as milligram of glucose taken up per gram of tissue. The bioethics committee approved all animal procedures and followed standard animal experimentation and care protocols.

Statistical analysis

In case of glucose adsorption, glucose diffusion, and amylolysis kinetics studies, data were expressed mean ± standard deviation (SD) of three replicate determinations. In case of glucose uptake studies using rat hemidiaphragms, values were expressed as mean ± SD ($n = 6$). Data

were subjected to analysis of variance using SPSS 20 SPSS ver. 20.0 (IBM, Armonk, NY, USA) and further subjected to Tukey's multiple comparisons *post hoc* test to distinguish between significantly different values. At the time, the values were considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

Glucose adsorption capacity of molokhia leaf powder

Figure 1 shows the glucose adsorption potential of WB and MLP. The results showed that the glucose-binding ability of WB and MLP increased as glucose concentration increased, with the maximum amount of glucose being adsorbed at 100 mM concentration. This observation is in good agreement with an earlier study wherein different dietary fiber sources such as oats, barley, and psyllium husk exhibited proportionate increase in glucose adsorption capacity with increasing molar concentration of glucose.[20] MLP (4%) was able to bind significantly more glucose ($P \leq 0.05$) than MLP (2%), but glucose adsorption ability of MLP at both 2% and 4% levels was significantly higher ($P \leq 0.05$) than WB at all glucose concentrations. This could be because WB primarily contains insoluble fibers whose water-holding capacity is lower than that of soluble gel-forming polysaccharide that is abundantly present in molokhia leaves.[8,20] Nevertheless, WB also adsorbed significant amount of glucose because soluble fibers, insoluble fibers, and resistant starch are all reported to bind glucose to varying degrees.[23-26] The soluble fibers are more effective in controlling postprandial hyperglycemia as they form viscous gel matrix which entraps glucose molecules and delay their diffusion across intestinal lumen.[27]

The effect of molokhia leaf powder diffusion of glucose through dialysis membrane

The diffusion of glucose across the dialysis membrane was measured every 60 min for 240 min in this study using WB (2%) used as reference. Table 1 shows the effect of various samples diffusion of glucose through dialysis membrane. When compared to control, MLP at 2% and MLP at 4% showed significantly slower glucose diffusion ($P \leq 0.05$) into dialysate through the dialysis membrane. At all time intervals, the glucose diffusion inhibitory effect of MLP was significantly high ($P \leq 0.05$) than that of WB. As shown by GDRI results, MLP at 4% inhibited glucose diffusion significantly better ($P \leq 0.05$) than MLP at 2%. The GDRI is an *in vitro* index that predicts how well a fiber can delay glucose absorption from the intestinal lumen.[28] The GDRI values for all samples decreased from 60 to 240 min in the current study, which is consistent with an earlier study in which the maximum GDRI values were found at 30 min and then decreased by 180 min for soluble fiber0rich foods – oats and psyllium husk.[29] The observed phenomenon in this study in relation to diminishing GDRI values over the study time can be attributed to the presence of mucilaginous polysaccharides present in molokhia leaves[8,21] as it is reported that fiber particle presents physical barrier toward movement of glucose molecules and also entraps glucose within fiber matrix.[28,30]

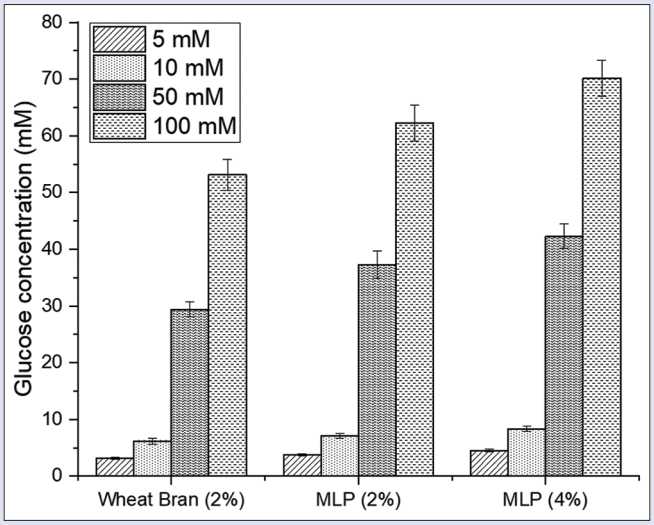

Figure 1: Glucose adsorption capacity of molokhia leaf powder. Values were represented as mean ± standard deviation (n = 3). MLP: Molokhia leaf powder

Furthermore, the increased viscosity caused by soluble polysaccharide slowed the movement of glucose into the dialysate through the dialysis membrane by adsorbing them directly within its fiber matrix.[26] Several studies have indicated that the ability of dietary components slow digestion of starch and absorption of glucose in the GI tract is linked to the degree of viscosity contributed by soluble dietary fiber.[31] Besides that, some investigators have also suggested that concentration and molecular mass of plant's soluble dietary fibers are major determinants of the hypoglycemic efficacy of particular plant.[32]

Molokhia leaf powders effect on amylolysis kinetics

In the present study, the ability of MLP to inhibit starch digestion and subsequent diffusion of glucose through dialysis membrane was studied using an *in vitro* model system. Table 2 shows the rate of glucose diffusion through the dialysis membrane and the GDRI values in the starch–amylase–fiber system. As compared to the control, the rate of glucose diffusion in the systems containing samples (MLP 2% and 4%) was significantly low ($P \leq 0.05$) at each interval of time. With acarbose, which resulted in complete inhibition of α-amylase, no glucose diffusion was observed at all time intervals, while no glucose diffusion was observed till 60 min in the system containing MLP (2%) and no glucose diffusion was observed till 120 min in the system containing MLP (4%). MLP (2% and 4%) inhibited glucose movement through the dialysis membrane significantly better ($P \leq 0.05$) than WB (2%). MLP at 4%, on the other hand, had a significantly high ($P \leq 0.05$) glucose diffusion retardation effect than MLP at 2%. WB, MLP (2%), MLP (4%), and acarbose (0.2%) had GDRI values of 22.3, 51.3, 63.5, and 100 at 240 min, respectively. Similar observations were reported by Bhinge *et al.*[33] wherein hydroethanolic extracts of *Musa sapientum* fruits exhibited significant hypoglycemic activity *in vitro* by decreasing glucose diffusion aided by increasing glucose adsorption. In another study, ethanol and aqueous extracts of *Caesalpinia bonducella* bark exhibited dose-dependent increase in glucose adsorption, leading to proportionate inhibition of glucose diffusion across dialysis membrane.[34]

The highest GDRI found with acarbose (a microbial origin oligosaccharide isolated from *Actinoplanes*) can be attributed to its strong α-amylase inhibitory effect.[35,36] The occurrence of both phenolic and non-phenolic compounds, potentially soluble polysaccharides in MLP, could be responsible to the observed retardation of glucose diffusion.[21] Molokhia leaves reportedly contain a diverse range of bioactive phytochemicals including vanillic acid, quinic acid, caffeic acid, ferulic acid, 4-O-caffeoylquinic acid, 3,4-di-O-caffeoyquinic acid, apigenin, apegenin-7-O-glucoside, naringin, naringenin, kaempferol, luteolin, p-coumaric acid, quercetin, myricetin, rutin, gingerol, protocatchuic acid, rosmarinic acid, cirsiliol, and cirsilineol.[21,37] Of these phytochemicals, caffeic acid, apigenin, kaempferol, luteolin, quercetin, and rutin are known to be potent inhibitors of α-amylase.[38-40] Since the polyphenol-rich aqueous extracts of *Corchorus* species did not show positive correlation with α-amylase inhibitory activity, it was

Table 1: Effect of molokhia leaf powder on glucose diffusion

Sample	Glucose content in the dialysate (mM) GDRI*			
	60 min	120 min	180 min	240 min
Control	1.25^{c}±0.05 (0.0)	1.70^{d}±0.09 (0.0)	2.03^{d}±0.07 (0.0)	2.25^{d}±0.09 (0.0)
Wheat bran (2%)	1.13^{b}±0.05 (18.30)	1.40^{b}±0.05 (16.1)	1.70^{c}±0.09 (13.91)	2.01^{c}±0.07 (11.10)
MLP (2%)	0.83^{d}±0.02 (36.95)	1.21^{c}±0.05 (26.11)	1.68^{c}±0.03 (15.01)	1.91^{c}±0.04 (13.30)
MLP (4%)	0.77^{c}±0.05 (42.51)	1.10^{a}±0.07 (37.10)	1.60^{b}±0.02 (17.05)	1.81^{b}±0.05 (17.31)

*P<0.05. Values were represented as mean±SD (n=3). Values with different superscript letters in columns differ significantly from each other at P≤0.05. Values in parenthesis represent GDRI. GDRI: Glucose dialysis retardation index; MLP: Molokhia leaf powder; SD: Standard deviation

Table 2: Effect of molokhia leaf powder on *in vitro* amylolysis kinetics

Sample	Glucose content in the dialysate (mM)/GDRI*			
	60 min	120 min	180 min	240 min
Control	0.31^{b}±0.03 (0.0)	0.47^{c}±0.04 (0.0)	0.64^{e}±0.05 (0.0)	0.81^{e}±0.04 (0.0)
Acarbose (0.2%)	0.00^{a}±0.00 (100)	0.00^{a}±0.00 (100)	0.00^{a}±0.00 (100)	0.00^{a}±0.00 (100)
Wheat bran (2%)	0.00^{a}±0.00 (100)	0.07^{b}±0.03 (73.1)	0.47^{d}±0.05 (31.1)	0.58^{d}±0.05 (22.3)
MLP (2%)	0.00^{a}±0.00 (100)	0.02^{a}±0.02 (96.0)	0.29^{c}±0.03 (65.1)	0.41^{c}±0.05 (51.3)
MLP (4%)	0.00^{a}±0.00 (100)	0.00^{a}±0.00 (100.0)	0.16^{b}±0.02 (83.7)	0.31^{b}±0.03 (63.5)

*P<0.05. Values were represented as mean±SD (n=3). Values with different superscript letters in columns differ significantly from each other at P≤0.05. Values in parenthesis represent GDRI. GDRI: Glucose dialysis retardation index; MLP: Molokhia leaf powder; SD: Standard deviation

hypothesized that α-amylase inhibitory activity of molokhia leaves is not only due to the presence of phenolics but also due to the presence of mucilaginous polysaccharides.[21] Thus, it is reiterated that the antidiabetic effect of molokhia leaves is mediated through delay of starch digestion aided by inhibition of α-amylase and entrapment of liberated glucose molecules through adsorption within soluble fiber matrix, which creates physical barrier for active glucose diffusion across intestinal lumen, thereby blunting postprandial hyperglycemia.[20,30,41]

Effect of molokhia leaf extract on rat hemidiaphragm glucose uptake

It is well documented that dietary components can exert antidiabetic effect not only by slowing down starch digestion and inhibiting its absorption but also by promoting glucose uptake in target tissues.[42] Determination of glucose uptake by rat hemidiaphragm is considered as an important technique to study peripheral glucose uptake because of the dynamic ability of diaphragm to utilize large amounts of glucose compared to other tissues. The technique employed in this study serves as a reliable indicator of *in vivo* antidiabetic effect.[43,44] The efficiency of MLE to facilitate glucose absorption in isolated rat hemidiaphragms was investigated in this study using *in vitro* system. The findings are summarized in Figure 2. Both insulin and MLE significantly improved ($P \leq 0.05$) the uptake of glucose by the hemidiaphragms, but no major variations with statistical significance were found between the glucose uptake enhancing effects of insulin and MLE. It is worth noting that MLE and insulin together exhibited complementary effect in enhancing glucose uptake which was higher than that of insulin alone but comparable with that of MLE alone. Previously, similar conclusions were drawn with extracts of *Ficus racemosa* and *Aegle marmelos*, wherein insulin and extracts had no synergistic effect on glucose uptake by the rat hemidiaphragm.[19,42] The increased glucose uptake/utilization by rat hemidiaphragm can conclusively be attributed to the occurrence of quercetin in MLE as an earlier study had shown that different fractions from ethanol extract of *Houttuynia cordata* plant significantly increased glucose uptake in isolated rat hemidiaphragm.[45] Bioactive fractions isolated from hydroethanolic extracts of the aerial parts of *Barleria prionitis* and *Hyptis suaveolens* have also been reported to exhibit dose-dependent enhancement of glucose transport in isolated rat hemidiaphragms.[46]

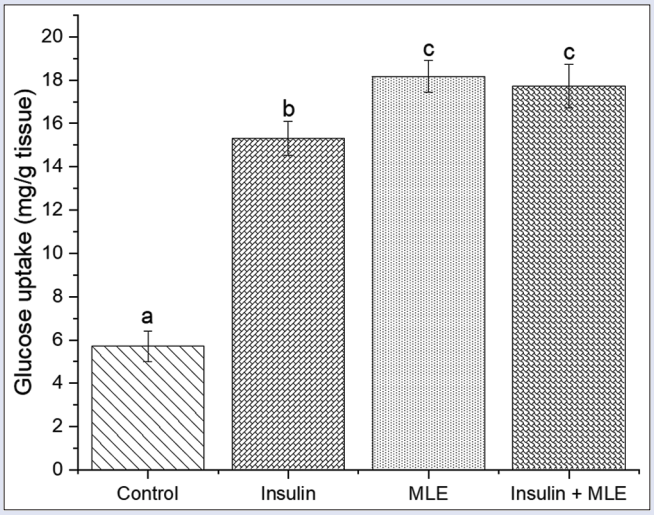

Figure 2: Effect of molokhia leaf extract on glucose uptake by isolated rat hemidiaphragm. Values were represented as mean ± standard deviation (n = 6). Bars with different superscript letters differ significantly from each other at $P \leq 0.05$. MLE: Molokhia leaf extract

CONCLUSION

Despite the fact that *in vitro* methods cannot predict exact *in vivo* antidiabetic potential of dietary components, the methods used in this study support the use of molokhia leaves as a diabetes adjunct. The findings conclusively show that molokhia leaves' antidiabetic effect is mediated by delaying starch digestion and physical adsorption of liberated glucose limiting its diffusion across intestinal lumen and enhancing glucose uptake in peripheral tissues, thereby aiding in reducing postprandial hyperglycemia.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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