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FULL PAPER

Protective effect of moringa-based beverages against hyperlipidemia and hyperglycemia in type 2 diabetes-induced rats

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Abstract

The objectives of this study were to determine the phenolic profile and potential antioxidant activity of *Moringa oleifera* leaves (MOL) cultivated in Saudi Arabia and to investigate the protective effect of moringa-based beverages on lipidemic and glycemic parameters in alloxan-induced diabetic rats (DR). The total phenolic content (TPC), chlorophylls, total carotenoids, total flavonoids, total flavonols, ascorbic acid content, and antioxidant activities (AOA) of MOL were determined and HPLC analysis of MOL was quantitatively performed. Moringa beverages as pure moringa (PM) and ginger-flavored moringa (GM) were given to diabetic rats (DR) at a dose of 1 mL 100 g^{-1} body weight/day for a successive six weeks. Glucose level (GL), lipid profile, and antioxidant parameters including malondialdehyde (MDA), reduced glutathione (GSH), and pancreas histopathological alterations were examined. Results indicated that MOL and prepared moringa-based beverages possess a suppressive amount of phenolics with considerable antioxidant activity *in vitro*. TPC was 78.72 mg GAE g^{-1} DW possess AOA as 36.33 µmol TE g^{-1} DW. Among the eleven identified phenolic acids and six flavonoids in MOL, caffeic acid and catechin were predominant and recorded 151.01 and 736.30 mg/100 g, respectively. Administration of PM or GM beverages significantly decreased GL and significantly improved serum lipid profiles in the DR group. Interestingly, oral administration of PM and GM beverages significantly attenuated the MDA and GSH levels in the DR compared to normal rats. Histopathologically, severe atrophy was noted in the islets of Langerhans in the DR group. However, after six weeks of oral administration of PM beverage, only mild atrophy was recorded in the islets of Langerhans, while with GM beverage, no histopathological alteration in the islets of Langerhans was found. The nutritional and biological evaluation of the moringa-based beverages revealed that both PM and GM beverages exhibit high antioxidant activity, possess antihyperglycemic and antihyperlipidemic effects, and alleviated alloxan-induced pancreatic damage in DR. Moringa could be recommended for diabetes and utilized for its great therapeutic benefits.

1. Introduction

Health concerns related to the ingestion of excessive calories involving high sugar consumption and association with diseases are considered critical issues by numerous organizations in the food industry (Weaver and Finke, 2003). A recent report on diabetes mellitus (DM) prevalence has indicated that the Kingdom of Saudi Arabia (KSA) ranks second in the Middle East and seventh in the world (WHO, 2017). Recently, the hypolipidemic, hypoglycemic, and hepatoprotective effects of different parts of the *M. oleifera* plant have been investigated (Paula *et al.,* 2017; Villarruel-López *et al.,* 2018; Oboh *et al.,* 2018; Balakrishnan *et al.,* 2019). *Moringa oleifera* Lam, belonging to the Moringaceae family, is a rapidly growing perennial foliage tree that is

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widely planted because of its great adaptability to climatic conditions (Teixeira *et al.,* 2014). It has been imported from the tropics to the sub-Himalayan regions, Latin America, Oceania, Africa, and tropical Asia (Fahey, 2005), and is successfully cultivated in KSA (Alaklabi, 2015; Mridha, 2015). Its nutritional, therapeutic, and prophylactic characteristics have resulted in *M. oleifera* being used as food, in medications, and for industrial purposes (Olayaki *et al.,* 2015).

Moringa oleifera leaves (MOL) contain substantial amounts of vitamins and considerable quantities of proteins, minerals, and phytonutrients (Amaglo *et al.,* 2010), as well as being a good source of total phenols (Hekmat *et al.,* 2015), with high antioxidant capacities (Paula *et al.,* 2017) for oral health care (Mohanty *et al.,* 2020). High amounts of phytochemicals such as kaempferol and quercetin glycosides were identified as the main phenolic compounds (Leone *et al.,* 2015). However, additional phenolics were characterized as gallic acid, syringic acid, quercetin 3-*O*-beta-glucoside, and rutin (Manguro and Lemmen, 2007). Many parts of the *M. oleifera* tree are recognized as being respectable sources of phenolic acids, flavonoids such as quercetin and kaempferol glucosides (Coppin *et al.,* 2013; Leane *et al.,* 2015), and glucosinolates (Amaglo *et al.,* 2010), carotenoids (Saini *et al.,* 2014a), polyunsaturated fatty acids (PUFAs), and folate (Saini *et al.,* 2016), as well as highly bioavailable minerals (Saini *et al.,* 2014b).

Another of the many therapeutic activities of *M. oleifera* is the reasonably high antioxidant capacity of its leaves, flowers, and seeds (Atawodi *et al.,* 2010). The potential therapeutic significance of *M. oleifera* against diabetes, rheumatoid arthritis, cancer, and other diseases have been reported (Gopalakrishnan *et al.,* 2016) as well as oxidative stress (Khalid *et al.,* 2020). The biological activities of *M. oleifera* include antiatherosclerotic (Chumark *et al.,* 2008), immune-boosting, antioxidant, and antimicrobial characteristics (Miyoshi *et al.,* 2004), anti cardiovascular disease, antiviral (Khalafalla *et al.,* 2010), and anti-inflammatory (Kumar Gupta *et al.,* 2013) properties, as well as tumor-suppressive effects in hepatocarcinoma cancer, colon cancer, and myeloma (Khalafalla *et al.,* 2010). Awodele *et al*. (2012) reported that the aqueous leaf extract of *M. oleifera* was safe when orally administered. Indeed, one goal in the KSA 2030 vision requires the updating of health-promoting research focusing on local and natural resources in particular. However, moringa is a newly cultivated plant in KSA (Alaklabi, 2015; Mridha, 2015) and research work concerned with it in the context of the rising incidence of DM is woefully inadequate (Abdulaziz Al Dawish *et al.,* 2016). Therefore, the present study aimed to investigate the bioactive components and phenolic profile of the newly cultivated *M. oleifera* in Gazan, KSA. To this end, flavored moringa-based functional beverages were prepared, and chemically and nutritionally evaluated. Furthermore, the hypolipidemic and hypoglycemic effects of these moringa functional beverages were investigated via biochemical and histopathological approaches.

2. Material and methods

2.1 Collection of Moringa oleifera leaves and Zingiber officinale rhizomes

Ten kilograms of dried *M. oleifera* Lam. leaves harvested in 2017 were obtained from the Al Owed Organic Nadawy Farm, Gazan, KSA. The leaves were inspected and stems and twigs, as well as yellow leaves, were removed. After cleaning, the leaves were kept at 4±1°C until used. Fresh ginger (*Z. officinale*) rhizomes were purchased from local markets in Buraidah, Al Qassim Region, KSA. The rhizomes were cleaned and kept at 4±1°C until extracted and processed.

2.2 Preparation of flavored moringa-based functional beverages and natural ginger distillate

The dried MOL was ground using a laboratory-type knife mill (Christison, CA, USA), extracted in boiling water (1:10, w:v) for 5 mins, and then immediately filtered through cheesecloth and divided into four lots. The first was divided into two portions. One of them was sweetened with sucralose (SU: BulkSupplements.com, 7511 Eastgate Road, Henderson, NV, USA) and formulated as pure moringa (PM) in a 3% sucralose base (PM+SU). The second portion was kept as it was (PM). The second lot was flavored with 2% ginger distillate and then divided into two portions. One of them was sweetened with SU and formulated as ginger-moringa (GM) in a 3% sucrose base (GM+SU) and the second was kept as it was (GM). The ginger distillate was prepared by thoroughly washing, cleaning, and slicing the ginger rhizomes (GR). The sliced ginger was mixed with water, boiled at 100°C for 10 min, and then cooled and slurred in a commercial laboratory blender (Christison Laboratory Blender, CA, USA) at low speed (18,000 rpm) for 2 mins. The slurry was filtered, steam distillation was carried out, and the distillate was collected and used as a natural ginger flavor.

2.3 Ascorbic acid determination

The ascorbic acid content in the dried MOL and moringa beverages was determined using the 2,6-dichloro phenol indophenol titrimetric method. The

ascorbic acid content was expressed as mg/100 g dw (Silva *et al.,* 1999).

2.4 Phytochemical analysis

Total phenolic compound (TPC) content was determined using the Folin-Ciocalteau method and was expressed as milligram of gallic acid equivalents per gram of sample (mg GAE 100 g^{-1} dw) according to Bettaieb *et al.* (2010). Total carotenoid (TC) content was determined colorimetrically as described by Yuan *et al.* (2009). The antioxidant activity as DPPH radical scavenging activity (DPPH-RSA) was determined colorimetrically using the 2,2-diphenylpicrylhy-drazyl (DPPH) radical. The DPPH free radical inhibition percentage was calculated; results were interpreted by plotting for the Trolox standard curve and were presented as µmol TE g^{-1} dw (Zhang and Hamauzu, 2004). Total flavonoids (TF) and total flavonols (TFL) contents were determined and results were presented as the mg quercetin equivalent (QE) g^{-1} using the method of Barakat and Ghazal (2016) and Kumaran and Karunakaran (2007), respectively.

2.5 Fractionation of phenolic compounds in M. oleifera leaves by (HPLC-DAD)

Defatted samples of moringa were dried in a hood at room temperature then extracted with 70% MeOH. The moringa extracts were evaporated under N stream and then resolved with absolute MeOH and analyzed using a liquid chromatography column (Agilent Technologies 1100 series) equipped with an autosampler and a diode-array detector (HPLC-DAD) (Kim *et al.,* 2006). The analytical column (Agilent Eclipse XDB C18, 150 × 4.6m; 5m, with a C18 guard column) used acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B) as the mobile phase. All samples were filtered through a 0.45-µm Acrodisc syringe filter (Gelman Laboratory, MI, USA) before injection. The run was performed at a flow rate of 0.8 mL min^{-1} for 70 mins, followed by 10 mins of post-run for reconditioning. Peaks were simultaneously monitored at 280, 320, and 360 nm. Phenolics were identified by congruent retention times and the UV spectrum, compared with known standards, and then integrated and calculated based on sample weight.

2.6 Biological evaluation of moringa beverages against diabetes-induced in rats

2.6.1 Animals

Male albino Wistar rats, weighing 150-180 g were housed at the Department of Food Science and Human Nutrition, College of Agriculture and Veterinary Medicine, Qassim University, KSA. The animals were allowed to become accustomed to the laboratory environment (23±2°C and 40-45% relative humidity) and the laboratory photoperiod cycle (12 hrs light: 12 hrs dark) for 15 days. The rats had free access to tap water *ad libitium*. All rats received a commercial diet covering recommended nutrient requirements. The current experimental protocol was approved by the Qassim University Animal Ethics Committee and met the guidelines for the care and use of animals in scientific research.

2.6.2 Experimental design and diabetes induction

Immediately after acclimation, the rats were separated into four groups of ten each. The first group (negative control) received distilled water as the vehicle, while the other three groups were intraperitoneally injected for three successive days with freshly prepared alloxan monohydrate solution (PubChem CID: 5781) at a dose of 150 mg kg^{-1} bw. One-week post-injection, rats showing fasting blood glucose of more than 200 mg dL^{-1} were considered as diabetic rats (DR) and were then divided into three groups. Group 2 (positive control) was kept as a pathogenically diabetic. Groups 3 and 4 were given the PM and GM beverages, respectively, in corresponding doses according to their fortnight weight. Beverages were administered through oral gavage in doses of 1 mL/100 g BW per day for a successive six weeks. The calculation was based on the consumption of 275 mL day^{-1} for a 70-kg human, as similarly reported by Rouanet *et al.* (2010). The body weight of the rats was measured at the beginning of the experimental period and then weekly for up to 6 weeks. At the end of the experiment and after an overnight fast, the rats were anesthetized and blood samples were collected from the jugular veins and then centrifuged at 3000 rpm for 30 mins to obtain blood serum, which was kept at –20ºC for biochemical analysis.

2.6.3 Blood biochemical and some antioxidant markers.

Glucose, triglycerides (TG), total cholesterol, and HDL-Cho were measured by commercially available kits. The LDL-Cho and VLDL-Cho were calculated according to Noda *et al.* (2000). Reduced glutathione (GSH) in the serum was estimated according to the method described by Beutler *et al.* (Beutler, 1963). The level of lipid peroxidation in the serum was determined by measuring malondialdehyde (MDA) formation at 534 nm using the thiobarbituric acid reactive substances (TBARS) method (Ohkawa *et al.,* 1979).

2.6.4 Histopathological examination.

At the end of the experiment, the rats were anesthetized using diethyl ether, bled, and then sacrificed. Necropsy samples were taken from the

pancreas of rats in the different groups and fixed in 10% formalin saline for 24 hrs. The fixed tissues were washed in cold saline buffer and gradually dehydrated using methyl, ethyl, and absolute ethyl alcohols. Specimens were prepared with xylene as a clearing agent and embedded into paraffin wax at 56°C for 24 hrs. The paraffin-beeswax tissue blocks were cut into 4-micron-thick sections using a sledge microtome. The tissue sections were fixed on glass slides, deparaffinized, and stained with Hematoxylin and Eosin for routine examination using a light electric microscope as described by Suvarna *et al.* (2018). Severity scoring of the histopathological alterations in photo sections of pancreatic tissues in each group was diagnosed blindly by two histopathological scientists according to Suvarna *et al.* (2018).

2.7 Statistical analysis

The statistical analysis was carried out using the SPSS software package (ver. 19) and analysis of variance (ANOVA) was applied according to the experimental design. The significance level was set as 0.05 and the Duncan test was performed according to Steel *et al.* (1997).

3. Results and discussion

3.1 Phytochemicals and antioxidant capacity of M. oleifera leaves and ginger rhizomes

The data in Table 1 demonstrate the TPC and antioxidant activity of the MOL and GR. In addition, phytochemicals such as chlorophyll *a* and *b*, TC, TF, and TFL, and antioxidant activity, as well as the ascorbic acid content of the MOL and GR are also given in Table 1. The TPC content was 78.72 and 388.3 mg GAE g^{-1} dw in the MOL and GR, respectively. As Moringa is a green leafy plant, the chlorophyll content was higher than in the ginger. The chlorophyll *a* content was 284.2 and 2.03 mg g^{-1} dw in the MOL and GR, respectively. The chlorophyll *b* content in the MOL and GR was higher than the chlorophyll *a* content. In contrast, the carotenoid content was higher in the GR than in the MOL, at 17.19 and 25.31 mg g^{-1} dw. *M. oleifera* was confirmed as a rich source of carotenoids (Zhang and Hamauzu, 2004; Saini *et al.,* 2014a; Saini *et al.,* 2014b). Similar results were recorded for flavonoids, flavonols, and vitamin C (Table 1). The *M. oleifera* leaves contained high levels of flavonoids and flavonols, e.g., quercetin and kaempferol (Coppin *et al.,* 2013; Amaglo *et al.,* 2010). The DPPH-RSA results for the MOL and GR referring to the Trolox equivalent g^{-1} [µmol TE g^{-1}] are given in the same table. The findings for antioxidant activity were 36.33 and 175.5 µmol TE g^{-1} dw for MOL and GR, similar to previous reports (Naeem *et al.,* 2012; Leone *et al.,* 2015). The results for TPC were higher and for antioxidant activity lower than those of Leone *et al.* (Leone *et al.,* 2015). The ascorbic acid in the MOL was higher than in the GR. A higher ascorbic acid content was observed than that found in current research. This may have been a result of dehydration (Saini *et al.,* 2014b). However, bioactive compound results, particularly for TPC, and antioxidant activity findings varied because of various factors (Naeem *et al*., 2012).

Table 1. Phytochemicals and antioxidant activity of *M. oleifera* leaves and ginger rhizomes

Items	Raw materials	
	MOL	GR
Total phenolic compounds [mg GAE g^{-1} dw]	78.72±5.74^{b}	388.3±13.59^{a}
Chlorophyll *a* [mg $g^{-1\ dw}$]	248.2±7.60^{a}	2.05±0.05^{b}
Chlorophyll *b* [mg $g^{-1\ dw}$]	496.39±15.2^{a}	4.16±0.10^{b}
Carotenoids [mg $g^{-1\ dw}$]	17.19±1.05^{a}	25.31±0.25^{b}
Flavonoids [mg QE $g^{-1\ dw}$]	22.76±0.44^{b}	56.87±1.59^{a}
Flavonols [mg QE $g^{-1\ dw}$]	13.5±0.13^{b}	49.1±4.71^{a}
Antioxidant activity [µmol TE $g^{-1\ dw}$]	36.33±1.62^{b}	175.5±9.88^{a}
Ascorbic acid [mg/100 g^{fw}]	175.14±5.66^{a}	60.15±2.78^{b}

MOL: *M. oleifera* leaves, GR: ginger rhizomes. Values are expressed as mean±SD. Values with different superscript within the same row are significantly different (P>0.05).

A high score for overall acceptability (based on color, odor, and bitter aftertaste) was recorded for the PM and GM beverages; however, the SU-sweetened beverages, in particular, were accepted at a confirmed higher rate than the unsweetened beverages (data not shown). The phytochemicals and antioxidant activity of the moringa (PM and PM+SU) and ginger-flavored moringa (GM and GM+SU) beverages are given in Table 2. The TPC content in the moringa beverages ranged from a low of 2.54 to 3.23 mg GAE 100 mL^{-1}. A significant difference was found between the (GM and GM+SU) and (PM and PM+SU) beverages. The chlorophyll *a* content was 36.39, 34.34, 32.05, and 35.13 mg 100 mL^{-1} in the PM, PM+SU, GM, and GM+SU, respectively. There was no significant difference (p>0.05) between the PM+SU and GM+SU, while a significant difference (p<0.05) was found between the PM and GM. The chlorophyll *b* content was 30.29, 27.74, 26.35, and 27.05 mg 100 mL^{-1} in the PM, PM+SU, GM, and GM+SU, respectively. In general, no significant difference (p>0.05) in chlorophyll *b* was found between the flavored and non-flavored moringa beverages. The carotenoid content was 0.39, 0.34, 0.33 and 0.38 mg 100 mL^{-1} in the PM, PM+SU, GM and GM+SU, respectively. Carotenoids were higher in the PM than in the GM, but no significant difference (p>0.05) was seen between the PM+SU and GM+SU;

Table 2. Phytochemicals and antioxidant activity of pure moringa and ginger-flavored moringa beverages

Items	Prepared beverages			
	PMB	PM+SU	GM	GM+SU
Total phenolic compounds [mg GAE 100 mL^{-1}]	2.9±0.02^{bc}	2.54±0.11^{c}	3.23±0.11^{a}	2.61±0.13^{ab}
Chlorophyll *a* [mg 100 mL^{-1}]	36.93±0.89^{a}	34.34±1.91^{ab}	32.05±1.03^{b}	35.13±1.02^{ab}
Chlorophyll *b* [mg 100 mL^{-1}]	30.29±0.58^{a}	27.74±1.87^{a}	26.35±1.13^{a}	27.05±0.70^{a}
Carotenoids [mg 100 mL^{-1}]	0.39±0.02^{a}	0.34±0.02^{ab}	0.33±0.02^{b}	0.38±0.01^{ab}
Flavonoids [mg QE 100 mL^{-1}]	0.94±0.05^{a}	0.85±0.02^{ab}	0.77±0.02^{b}	0.77±0.03^{b}
Flavonols [mg QE 100 mL^{-1}]	0.62±0.01^{a}	0.57±0.04^{a}	0.63±0.00^{a}	0.59±0.00^{a}
Antioxidant activity [µmol TE 100 mL^{-1}]	1.13±0.07^{b}	1.21±0.01^{b}	1.26±0.01^{ab}	1.25±0.04^{ab}
Ascorbic acid [mg 100 mL^{-1}]	1.52±0.05^{ab}	1.34±0.05^{b}	1.59±0.05^{a}	1.50±0.07^{ab}

PM: pure moringa beverages, PM+SU: pure moringa beverages sweetened with sucralose in 3% sucrose base, GM: ginger-flavored moringa beverages, GM+SU: ginger-flavored moringa beverages sweetened with sucralose in 3% sucrose base . Values are expressed as mean±SD. Values with different superscript within the same row are significantly different (P>0.05).

however, a significant difference between the PM and GM was noted. The added ginger extract in the GM may have caused a slight dilution in the carotenoid content. The flavonoid content was 0.94, 0.85, 0.77, and 0.77 mg QE 100 mL^{-1} in the PM, PM+SU, GM, and GM+SU, respectively. A significant difference (p<0.05) was found between the PM and GM. In contrast, the flavonol content was 0.62, 0.57, 0.63, and 0.59 mg QE 100 mL^{-1} in the PM, PM+SU, GM, and GM+SU, respectively. No significant difference (p>0.05) in flavonol content was observed among the prepared moringa beverages (Table 2). The antioxidant activity values were 1.13, 1.21, 1.26, and 1.25 µmol TE 100 mL^{-1} in the PM, PM+SU, GM, and GM+SU, respectively. There was no obvious significant difference (p>0.05) among all the prepared beverages. The vitamin C values were 1.52, 1.34, 1.59, and 1.50 mg 100 mL^{-1} in the PM, PM+SU, GM, and GM+SU, respectively. Interestingly, manufacturing such products would present beverages with valuable bioactive contents that could provide various functional features. The raw moringa and ginger content was retained and was determinable in the beverages. These findings are closely in accordance with those of Manguro *et al*. (2015), Zhang *et al*. (2004), and are confirmed by Badejo *et al.* (2014).

3.2 Phenolic compound fractionation of Moringa oleifera leaves

The data in Table 3 illustrates the simultaneous HPLC quantitative analysis of the phenolic compounds (mg/100 g) of MOL as the main component of the prepared beverages. The phenolic compounds were analyzed via HPLC and showed that MOL is rich in phenolic compounds. Twenty-two standards were applied for the potential occurrence of phenolics in methanolic extracts of MOL. Seventeen phenolic compounds were identified in the MOL extract. Among the eleven phenolic acids, caffeic was the predominant phenolic acid in the MOL extract at a high concentration of 151.01 mg/100 g, while rosmarinic acid was the lowest (Table 3). The protocatechuic, *p*-hydroxybenzoic, gentisic, syringic, vanilic, ferulic, sinapic, *p*-coumaric, and cinnamic were 4.91, 19.86, 6.97, 3.12, 3.33, 7.68, 0.78, 1.77, and 1.79 mg/100 g, respectively. Among the six flavonoids, catechin was represented at a high concentration of 736.30 mg/100 g, followed by luteolin at 348.85 mg/100 g, and rutin at 42.35 mg/100 g. The obtained phenolic profile showed mixed results as the effects on phenolics were based on various factors (Naeem *et al.,* 2012). Indeed, many studies have been concerned with the diversity of the phenolic profile and related antioxidant activities of MOL (Kim *et al.,* 2006; Manguro and Lemmen, 2007; Saini *et al.,* 2014a; Leone *et al.,* 2015).

Table 3. Quantification analysis of phenolic compounds in *M. oleifera* leaves using HPLC-DAD

Compound *	Retention time	mg 100 g$^{-1\ DW}$
Phenolic acids		
Protocatechuic	7.59	4.91
p-hydroxybenzoic	11.00	19.86
Gentisic	11.84	6.97
Caffeic	14.54	151.01
Syringic	15.39	3.12
Vanillic	17.19	3.33
Ferulic	26.44	7.68
Sinapic	28.28	0.78
p-coumaric	32.58	1.77
Rosmarinic	38.01	0.42
Cinnamic	41.33	1.79
Flavonoids		
Catechin	12.60	736.30
Rutin	30.87	42.35
Apigenin-7-glucoside	36.83	1.09
Luteolin	44.88	348.85
Apigenin	48.16	0.85
Kaempferol	48.76	2.25

* Phenolic acids and their derivatives were detected at 280–320 nm. Flavonoids were detected at 360 nm. DW: Dry Weight.

3.3 Effect of oral administration of Moringa olefiera beverages on weight gain of diabetic rats

Figure 1 demonstrates the effect of the oral administration of PM and GM beverages on the weight gain percentage of diabetic rats (DR). After one week, the weight gain percentage ranged from 8.95% in the DR to 17.54% in the normal rats (NR). A significant difference was found between the NR and the DR, DR+PM, and DR+GM for up to 6 weeks. Administration of PM or GM beverages to the DR significantly improved their weight gain results. Regarding the mean period, there was a significant difference (p<0.05) between the NR and the treated rats. No significant difference was noted between DR+PM and DR+GM, which both differed significantly from the NR group. These results showed a similar trend more or less related to the results obtained by Olayaki *et al.* (2015), Toma *et al.* (2015), and Balakrishnan *et al.* (2018).

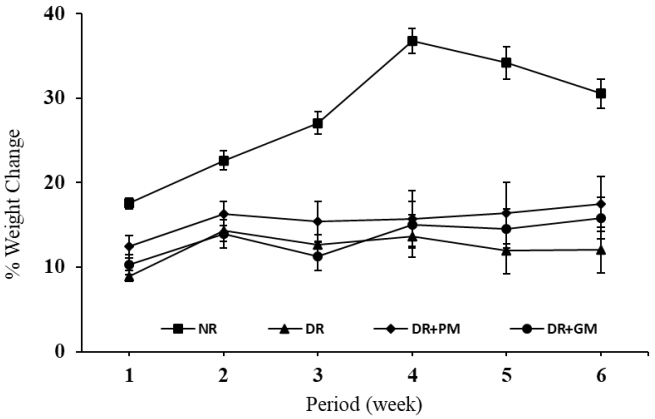

Figure 1. Effect of oral administration of moringa beverages on weight gain percentage of diabetic rats. NR: normal rats (negative control), DR: diabetic rats (alloxan-injected), DR+PM: diabetic rats administrated moringa, DR+GM: diabetic rats administrated ginger-flavored moringa.

3.4 Effect of oral administration of Moringa olefiera beverages on the lipid profile of diabetic rats.

Table 4 illustrates the effect of oral administration of moringa beverages on the lipid profiles including triglycerides, total cholesterol, HDL-Cho, LDL-Cho, and VLDL-Cho of the DR. The triglycerides ranged from 40.67 mg dL^{-1} in the NR to 83.67 mg dL^{-1} in the DR. As a complication of diabetes, the triglycerides increased significantly (Tuorkey, 2016; Villarruel-López *et al.,* 2018). A significant difference (p<0.05) was found between the DR and the NR, DR+PM, and DR+GM. The total cholesterol ranged from 59.67 mg dL^{-1} in the NR to 77.33 mg dL^{-1} in the DR, whereas, because of the alloxan injections, increases in total cholesterol were noted after 6 weeks. Administration of PM reduced the total cholesterol, whereas the GM treatment was much better, and resulted in only a non-significant difference between the DR and the NR groups. A significant difference (p<0.05) in the HDL-Cho content was seen between the non-treated and treated DR rats. Diabetes induction reduced the HDL-Cho significantly (Table 4). However, the LDL-Cho was represented by a high value in the DR group, which differed significantly (p<0.05) from the NR group. A significant reduction of the LDL-Cho level in the moringa-treated groups was observed. In the same context, compared to the treated groups, the VLDL-Cho in the DR increased significantly (p<0.05). There was no significant difference (p<0.05) in the VLDL-Cho level among the moringa-treated groups and the NR. These results showed a trend similar to that seen in the results obtained by Olayaki *et al.* (2015), Tang *et al.* (2017), Balakrishnan *et al.* (2018), Villarruel-López *et al.* (2018), and Toma *et al.* (2015).

3.5 Effect of oral administration of Moringa olefiera beverages on glucose level, MDA, and GSH of diabetic rats.

The data in Table 5 and Figure 2 demonstrate the effect of the oral administration of moringa beverages on glucose levels and MDA and GSH contents in the serum of the diabetic rats. Over the 6 weeks, the fasting glucose level showed a higher value in the DR group when compared to the NR group. Administration of PM and GM decreased the glucose level significantly, approaching very close to that of the NR group. These results showed a trend similar to other findings obtained recently (Chumark *et al.,* 2008; Paula *et al.,* 2017).

As an oxidation indicator, MDA was determined in

Table 4. Effect of oral administration of moringa beverages for 6 weeks on the lipid profile of diabetic rats (n = 10)

Treatment	Parameters (mg dL^{-1})				
	Triglycerides	Total Cholesterol	HDL-Cho	LDL-Cho	VLDL-Cho
NR	40.67±4.81^{a}	59.67±3.28^{b}	33.33±2.88^{a}	18.20±0.78^{c}	8.13±0.96^{b}
DR	83.67±5.43^{b}	77.33±7.06^{a}	22.03±4.25^{b}	40.57±2.49^{a}	14.73±1.09^{a}
DR+PM	43.17±4.96^{a}	71.73±5.51^{a}	34.17±2.90^{a}	28.93±3.83^{b}	8.63±0.99^{b}
DR+GM	44.67±4.75^{a}	64.96±4.29^{ab}	30.01±3.20^{a}	26.03±1.88^{b}	8.93±0.95^{b}

NR: normal rats (negative control), DR: diabetic rats (alloxan-injected), DR+PM: diabetic rats administrated moringa, DR+GM: diabetic rats administrated ginger-flavored moringa . Values are expressed as mean±SD. Values with different superscript within the same row are significantly different (P>0.05).

all groups (Table 5). The data indicated that the MDA level was significantly higher in the DR group than in the NR group, which confirms the diabetes complications. Subsequently, the alloxan injection decreased the GSH significantly, up to 10.04 µmol L^{-1} in the DR group, compared to 23.07 µmol L^{-1} in the NR. Oxidative stress is known to be associated with diabetes and its complications (Jaiswal *et al.,* 2013). In the present study, compared to the NR, a significant increase in the MDA level was observed as a biomarker of oxidative stress in the serum of the alloxan-induced DR. Lipid peroxidation is harmed by MDA, which is a component capable of eliciting much damage to the body and brain (Long *et al.,* 2009). In this study, the MDA level increased and the GSH level concomitantly decreased in the DR group because of the induction. Administration of PM and GM decreased the MDA significantly compared with both the NR and DR groups. In the same context, the administration of PM and GM raised the GSH significantly in the DR+PM and DR+GM groups. Our findings revealed that moringa beverages improved the antioxidant status through modulation of the MDA level, antioxidant enzyme activities, and an increase in the GSH level (Jaiswal *et al.,* 2013; Tang *et al.,* 2017; Balakrishnan *et al.,* 2019) which can be attributed to the phenolic compounds and especially to the higher content of caffeic, catechin, luteolin, and rutin (Table 3). Phenolics are sturdy antioxidants that possess considerable active radical scavenging ability and quench singlet oxygen, as well as activating antioxidant enzymes. The observed improvement in the enzymatic and non-enzymatic antioxidant defense systems exhibited by the PM and GM beverages could protect the body from oxidative stress. These results are similar in trend to the results attained by Tang *et al.* (2017), Oboh *et al.* (2018), and Balakrishnan *et al.* (2019).

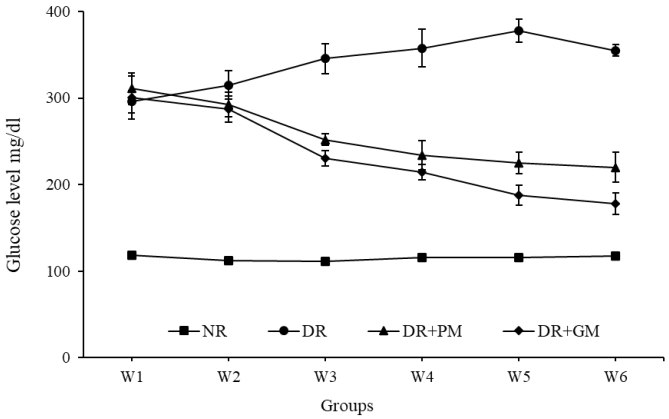

Figure 2. Effect of oral administration of moringa beverages for 6 weeks on non-fasting glucose level in blood during the experiment. NR: normal rats (negative control), DR: diabetic rats (alloxan-injected), DR+PM: diabetic rats administrated moringa, DR+GM: diabetic rats administrated ginger-flavored moringa.

Table 5. Effect of oral administration of moringa beverages for 6 weeks on fasting glucose, MDA, and GSH profile of diabetic rats (n = 10)

Treatment	Glucose (mg dL^{-1})	MDA (nmol mL^{-1})	GSH (µmol L^{-1})
NR	60.67±4.17^{c}	0.18±0.01^{d}	23.07±0.64^{a}
DR	152.17±13.10^{a}	0.51±0.01^{a}	10.04±0.28^{d}
DR+PM	89.83±11.31^{b}	0.36±0.01^{b}	16.77±0.58^{c}
DR+GM	79.55±12.44^{b}	0.24±0.02^{c}	20.61±0.47^{b}

NR: normal rats (negative control), DR: diabetic rats (alloxan-injected), DR+PM: diabetic rats administrated moringa, DR+GM: diabetic rats administrated ginger-flavored moringa. Values are expressed as mean±SD. Values with different superscript within the same row are significantly different (P>0.05).

3.6 Histopathological alterations in the pancreas of normal and alloxan-induced diabetic rats after treatment with moringa beverages

The histopathological findings of the pancreas in normal (negative control) and diabetic rats treated by moringa beverages are presented in Figure 3, I-IV. No histological alteration was observed and the regular histological structures of the endocrine islets of Langerhans as well as the exocrine acini are illustrated in Figure 3 I. However, severe atrophy can be noticed in the islets of Langerhans as shown in Figure 3, II. This histo-architectural study of the pancreas in alloxan-induced diabetic rats displays a decrease in the volume of the islets, vast necrotic changes, damaged β-cell populations, fibrosis, and atrophy (Toma *et al.,* 2015; Omodanisi *et*

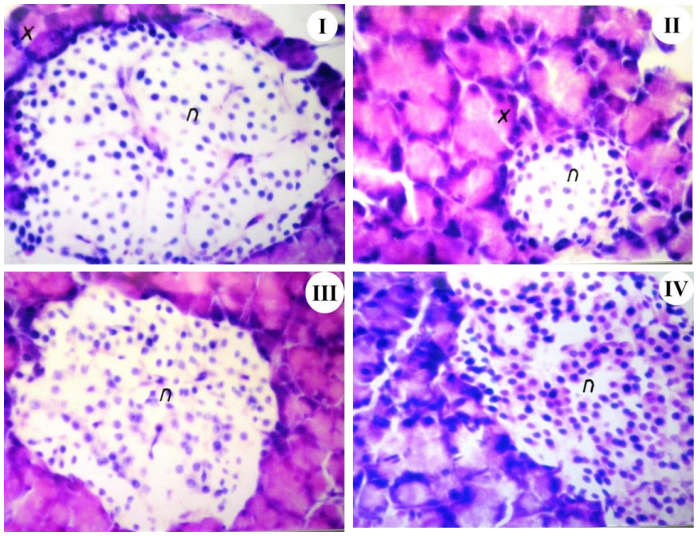

Figure 3. The histopathological findings of experimental groups: (I) No histopathological alteration was observed and normal histological structures of the endocrine islets of Langerhans as well as the exocrine acini were recorded, x = 100, (II) Sever atrophy was seen in the islets of Langerhans with vast necrotic changes and remarkable fibrosis, x = 100, (III) A mild atrophy was seen in the islets of Langerhans, x = 100, (IV) No histopathological alteration was observed in the islets of Langerhans, x = 100.

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Table 6. Severity scoring of the histopathological alterations in pancreatic tissues of normal and Alloxan-induced diabetes and treated diabetic rats with PM and GM after 6 weeks (n = 10)

Alteration items	Experimental groups			
	NR	DR	DR+PM	DR+GM
Atrophy of islands of Langerhans	-	+++	+	-
Absence of Langerhans cells	-	+++	-	-
Ductal hyperplasia	-	++	-	-

+++ = Sever, ++ = Moderate, + = Mild, - = Nil. NR: Normal rats, DR: Alloxan-diabetic rats, DR+PM: Diabetic rats + PM beverage and DR+GM: Diabetic rats + GM beverage.

al., 2017). With oral administration of PM for 6 weeks, mild atrophy in the islets of Langerhans can be observed (Figure 3, III). The PM attenuated the fibrotic and necrotic alterations and improved the number and size of the islets. Interestingly, with the administration of the GM beverage, no histopathological alteration was revealed in the islets of Langerhans, as recorded in Figure 3, IV. The severity scoring of the histopathological alterations in the pancreatic tissues of all experimental groups is given in Table 6. The attained results were in agreement with those of Tang *et al.* (2017), Oboh *et al.* (2018), Balakrishnan *et al.* (2019), and Toma *et al.* (2015).

4. Conclusion

People usually select beverages based on several features. Under the circumstance of illness, the health impact is considered when choosing beverages. To attenuate the buildup of oxidative stress in the body, attention needs to be given to plant-based foods, especially those that are grossly underutilized. In conclusion, the present study confirmed that the moringa plant is rich in TPC with highly relevant antioxidant activities. Interestingly, oral administration of PM and GM beverages significantly reduced the blood glucose level and improved the lipid profile in alloxan-induced diabetic rats. These properties validate the possible useful effects of *M. oleifera* consumption in diabetes and encourage further studies to gain an understanding of its relevant mechanisms of action as an antidiabetic agent. Indeed, moringa has recently been cultivated in KSA, and more studies on moringa and its products are needed. Functional beverages containing MOL extracts mixed with GR are laden with antioxidative and antidiabetic potential. The consumption of these beverages in different forms should be encouraged.

Conflict of interests

The authors declare no competing interests.

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