Indian Journal of Natural Products and Resources
Vol. 13(2), June 2022, pp. 183-187
DOI: 10.56042/ijnpr.v13i2.33499

Effect of the leaf and seed extract of *Moringa peregrina* (Forssk.) Fiori. in alloxan induced diabetic Mice

Salla Hemadri Reddy*, Mizna Salim Ali Al Hatmi and Alshima Rashid Al Kalbani

Applied Biology Section, Department of Applied Science, Higher College of Technology, Al Khuwair, Postal code 117, Muscat, Sultanate of Oman

Received 07 April 2020; Revised 02 February 2022

Moringa peregrina (Forssk.) Fiori. is a well known medicinal plant used widely in the Sultanate of Oman for the treatment of various disorders and diseases. In the present study, the antidiabetic potential with relevance to net weight change was carried out using leaf and seed extracts. The experiment was carried out in alloxan (150 mg/kg body weight) induced diabetic mice by oral administration (1.5 g/kg body weight) of *M. peregrina* seed and leaf extracts for 21 days. Pre and post treatment sugar levels and body weight were monitored and evaluated. The results of antidiabetic activity of the extracts exhibited a significant effect on reducing the blood glucose level in diabetic mice and showed very low effect on the mice body weight. Among the tested extracts against diabetes in mice, the seed extract showed a significant reduction of blood sugar (-101.4 mg/dL; $P \leq 0.001$) followed by leaf extract (-83.7 mg/dL; $P \leq 0.001$) without affecting body weight. These findings suggest that the hypoglycaemic effect of *M. Peregrina* seed can be further studied to evaluate its mode and mechanism of action in the regulation of blood sugar levels as well the chemical composition in the seed extracts need to be studied.

Keywords: Alloxan, Antihyperglycaemic, Blood glucose, Body weight, Diabetes, Hypoglycaemic, *Moringa peregrina*.

IPC code; Int. cl. (2021.01)-A61K 36/00, A61K 127/00, A61K 131/00, A61P 3/00, A61P 3/10,

Introduction

Globally, the number of people with diabetes and pre-diabetes is increasing exponentially, mainly because of old age, urbanization, unhealthy eating habits, increasing obesity prevalence and lack of physical activity[1]. Diabetes mellitus was one of the world's leading causes of disease and death, affecting an estimated 382 million adults and killing 5.1 million in 2013. Herbal medicines with lowering effects of blood glucose were used as an alternative treatment for type 2 diabetes mellitus in the increasing interest to improve quality and healthy life[2]. Continuous efforts are underway throughout the globe to study and identify various factors which are responsible for diabetes as well as the management and understanding of diabetes. The disease and disease related complications with diabetes are increasing continuously due to multiple defects in physiology[3]. The available options for the treatment of Diabetes include strict dietary habits, the use of insulin or oral hypoglycaemic drugs (sulfonylureas, biguanides, and thiazolidinediones). Numerous complications are common incidents of the disease and the mortality index due to the usage of synthetic drugs leads to many side effects[4]. New drugs/compounds with affordable price with fewer or no side effects are needed to be screened.

The majority of the population in developing countries depend on medicinal plant products for primary health related problems since herbal medicine is available in plenty and affordable[5]. The World Health Organization (WHO) is also interested in the development of hypoglycaemic agents from medicinal plants as herbal natural remedies to treat diabetes mellitus due to their being cost-effective and safe[6]. Unfortunately, herbal medicines have yet to be proven scientifically with evidence, especially with respect to the physiology of diabetes therapy. Studies on the plants with antidiabetic activity will definitely confer scientific and systematic approach for the use of these plants as hypoglycaemic agents. Due to the wide traditional applications, the research aims to study the antidiabetic property and its physiological role on the weight of diabetes induced mice with *Moringa peregrina* (Forssk.) Fiori used in Sultanate of Oman for traditional healing of many diseases[7]. Recently, *M. Peregrina* has gained more importance due to its

*Correspondent author
Email: salla.reddy@hct.edu.om

medicinal, traditional, industrial, and nutritional values. Since this plant has a wide range of traditional importance, it has been studied for various pharmacological activities in the past years[8].

M. peregrina is commonly known as horseradish tree or drum stick tree in English and Habb Elyasar or Yen in Arabic, which grows uncontrollably in Oman. It is available in many zones of the world like North-eastern tropical Africa, Madagascar, and Arabia. *M. peregrina* is a deciduous tree belonging to the family of *Moringaceae*. It is a fast growing tree among the other Moringa species[9] with 3–10 m height and grayish green bark adapted to high aridity. The leaves are 30–40 cm long, alternate, obovate and deciduous. The leaves are 30–40 cm long, alternate, obovate and deciduous. One of the unique features of *M. peregrina* is the falling of their leaflets when the leaves mature, leaving leaf rachises naked[10-11]. In Northern Oman, oil extracted from the pods are utilized for the treatment of infantile paralysis traditionally. Due to the presence of numerous phytoconstituents, *M. Peregrina* can be used as medicine, food, water purifying retailers, and biodiesel agent[12]. *M. Peregrina* seed oil contains around 70% oleic acid and all other unsaturated fats that are found in olive oil. A study[13] reported that the unsaturated fatty acids present in the *M. peregrina* are as follows: palmitic 9.3%, palmitoleic 2.4%, stearic 3.5%, oleic 78.0%, linoleic 0.6%, linolenic 1.6%, arachidic 1.8% and behenic 2.6%. The pharmacological activities of *M. peregrina* leaves revealed that it has antimicrobial, anticancer, antioxidant, and pain relieving properties[14]. Therefore the current research is aimed to investigate the antidiabetic property to evidence scientifically the herbal usage and to further explore its complete utilization.

Materials and Methods

M. peregrina leaves and seed, Soxhlet apparatus, Alloxan monohydrate- Aldrich (BCBN9150V), Glucose, n-hexane, Metformin hydrochloride, Healthy Male Mice, Glucometer-ONETOUCH Select plus, Dimethyl sulphoxide(DMSO).

Sample collection and identification

M. peregrina leaves and seed were collected from Rustaq (23.4425° N, 57.4344° E) and Ibri (23.2359° N, 56.4944° E), Sultanate of Oman in January 2019. The plant material was identified by Dr Pankaj Sah, Botanist, Applied Biology Section, Department of Applied Science, HCT, Oman by using the book "Handbook of Arabian Medicinal Plants"[15]. The collected materials were processed and stored in the refrigerator at 4 °C for 48 h and continued the extraction process.

Preparation of n-hexane leaf and seed extracts of *M. peregrina*

The leaf and seed of *M. peregrina* were washed in tap water and oven dried at 40 °C for 48 h. The leaves and seeds were chopped into small pieces and grounded into a fine powder using a blender and extracted using soxhlet apparatus with *n-hexane*.

Experimental animals

Thirty (30) male mice weighing between 20 to 25 g were used to study the potential of *M. peregrina* on anti-hyperglycemia in alloxan-induced diabetic mice. The animals were obtained from UTAS-Higher College of Technology, Animal House and were maintained under standard conditions of temperature and diet. The ethical clearance (ECA: 2018-2019/II/APS/BIO/B TECH/1) has been obtained from biosafety and ethical committee of Applied science department, Higher college of Technology, Oman. All animals were caged into 5 groups (6 mice/cage) and allowed free access to standard laboratory food and water, 7 days before starting the experiment and during the period of the experiment. All animals were fed with pelletized diet and water *ad libitum.*

Antidiabetic study

The antidiabetic study was carried out by the method of Salla *et al.*[16] with slight modifications based on sample materials used in the present experiment.

Induction of diabetes

Diabetes was induced in the mice by a single intraperitoneal injection of freshly prepared alloxan monohydrate of 150 mg/kg body weight in normal saline[17]. Twenty four hours after alloxan administration, blood samples were collected from the tips of the mice tail and the fasting blood glucose levels were determined using OneTouch Ultra glucometer (Life Scan, Milpitas, CA, USA) to confirm diabetes.

Animal grouping

Mice were divided randomly into 5 groups as follows: Group 1 is Non diabetic control mice received distilled water, Group 2 is Untreated diabetic mice received distilled water, Group 3 is Diabetic control mice received metformin (5 mg/kg body weight), Group 4 is Diabetic mice received 400 mg/kg

body weight of leaf extract of *M. peregrina*, Group 5 is Diabetic mice received 400 mg/kg body weight of seed extract of *M. peregrina*.

Blood sugar analysis

In each cage, 6 healthy male mice were maintained, and treatment with the respective extract was carried out for 21 days. For determination of blood glucose level, blood samples of mice were drawn after overnight fasting (12 h) from the tail vein before and after 21 days of treatment. Along with blood sugar analysis, the mice body weight was also determined to study the influence of blood sugar levels on body weight.

Statistical analysis

Data were analyzed using descriptive (mean±SE) and comparative statistics (paired t-test, $P \leq 0.05$) by Microsoft Excel v. 2010.

Results

Effect on blood sugar

There was an increase in the blood sugar with alloxan monohydrate injected intraperitoneally, which was reduced significantly by the oral administration of *M. peregrina* seed and leaves extracted with n- hexane (Table 1). The normal blood sugar level ranges from 80-120 mg/dL under normal physiological condition. Alloxan-induced diabetic mice with blood sugar of 211.1±4.73 mg/dL, when treated with *M. peregrina* leaf extracts in n-hexane (400 mg/kg/day) for 21 days, decreased to 127.4±17.25 mg/dL with a net difference of -83.7 mg/dL (t = 6.38). Alloxan-induced diabetic mice with blood sugar at 207.8±1.92 mg/dL, when treated with *M. peregrina* seed extracts in n-hexane (400 mg/kg/day) for 21 days, decreased to 106.4±11.30 mg/dL with a net difference of -101.4 (t = 9.56). Among the seed and leaf extracts tested seed extract has the highest potential in reduction (101.4 mg/dL: t = 9.56) in blood sugar level in alloxan-induced diabetic mice in comparison with leaf extract (83.7 mg/dL: t=6.38). Whereas the alloxan induced diabetic mice (untreated) showed increasing the blood sugar from 201.8±0.70 to 236.4±8.62 mg/dL with net increase 34.6 mg/dL (t= -4.27). The diabetic mice treated with metformin the positive control showed reduction in blood sugar level from 200.5±2.26 to 111.6±7.29 mg/dL with a net difference 88.9 mg/dL (t=12.24).while the untreated non diabetic mice blood sugar remain same with a slight increase from 118.5±4.16 to 114.3±5.82 mg/dL with a difference -4.2 (t= 1.87).

Effect on body weight

The alloxan-induced diabetic mice treated with different extracts were studied to determine the effect of diabetes on body weight (Table 2). The untreated diabetic mice didn't show any significant weight loss or gain. On the 1st day of diabetic induction, the mice weight was 25.6±0.75 g while after 21 days it was 25.3±1.04 g with a net loss 0.3 g (t= 0.24). The

Table 1 — Effect of leaf and seed extracts of *M. peregrina* on blood sugar level in diabetic mice

Groups	Day 1 blood sugar (mg/dL)	Day 21 blood sugar (mg/dL)	Net difference (mg/dL)	t test	*P* value
Group I	118.5±4.16	114.3±5.82	-4.2	1.87	0.06
Group II	201.8±0.70	236.4±8.62	+34.6	-4.27	0.003
Group III	200.5±2.26	111.6±7.29	-88.9	12.24	3.21
Group IV	211.1±4.73	127.4±17.25	-83.7	6.38	0.0006
Group V	207.8±1.92	106.4±11.30	-101.4	9.56	0.0001

Note: values are mean±SE of 6 individuals (*n*=6), paired t-test analysis was carried out between before (day 1) and after treatment (21st day) of individuals of each experiment (df=5).

Table 2 — Effect of leaf and seed extracts of *M. peregrina* on body weight in diabetic mice

Groups	Day 1 body weight (g)	Day 21 body weight (g)	Net difference (g)	t test	*P* value
Group I	24.4±0.38	24.9±0.65	+0.5	-1.01	0.17
Group II	25.6±0.75	25.3±1.04	-0.3	0.24	0.40
Group III	24.6±1.22	27.2±0.84	+2.6	-6.37	0.0007
Group IV	22.7±0.82	21.3±0.83	-1.4	1.66	0.07
Group V	21.6±0.79	22.2±0.76	+0.6	-1.22	0.13

Note: values are mean±SE of 6 individuals (*n*=6), paired t-test analysis was carried out between before (day 1) and after treatment (21st day) of individuals of each experiment (df=5).

alloxan-induced diabetic mice treated with metformin, the positive control showed an increase in the body weight. The weight of mice on the 1st day was 24.6±1.22 g while after treatment for 21 days the weight was 27.2±0.84 g with a net gain 2.6 g (t= -6.37). The normal mice which are non diabetic and untreated, showed similar results in gaining slight weight. On the 1st day of experiment, the mice weight was 24.4±0.38 g while after 21 days, the weight was 24.9±0.65 g with a net gain 0.5 g (t= -1.01).

The alloxan-induced diabetic mice with body weight on the 1st day of treatment was 22.7±0.82 g, when treated with *M. peregrina* leaf extracts in n-hexane (400 mg/kg/day) for 21 days, decreased to 21.3±0.83 g with a net loss of 1.4g (t= 1.66). The alloxan-induced diabetic mice with the body weight on the 1st day of treatment was21.6±0.79 g, when treated with *M. peregrina* seed extracts in n-hexane (400 mg/kg/day) for 21 days, increased to 22.2±0.76g with a net gain of 0.6 g(t= -1.22; *P*=0.13). Among the tested samples against body weight in diabetic mice, metformin treated group showed highest gain 2.6 g and lowest gain 0.6 g by seed extract treated group. While the other groups showed decrease in body weight.

Discussion

Herbal medicine is the traditional form of healthcare known to humankind for many decades, in which plants seeds, berries, roots, leaves, bark (stem), or flowers are used for medicinal purposes. Using these materials in the treatment/management and prevention of diseases is the main course in traditional medicine, especially diabetes mellitus[18]. The presence of phenolic rich substances in the medicinal plants might be the reason for controlling or managing diabetes mellitus since these phytoconstituents are directly responsible for the antioxidant properties in which reactive oxygen species responsible for damaging the insulin producing cells will be protected. Since free radicals are responsible for damaging and causing pathogenicity in β cells of pancreas. The radical scavenging abilities of the phenol-rich extract of *M. peregrina* leaves and seed extracts could be a reference point in the management of diabetes mellitus, it is reported[19-20] that *M. peregrina* is rich in phenols, flavonoids and many more biologically active substances[21]. The traditional usage of seed and oil extracts is evidenced scientifically that the *M. peregrina* is effective in the treatment of diabetes mellitus.

The results of antidiabetic study of *M. peregrina* seed and leaf showed significant reduction in blood sugar levels when compared with non treatment groups. The result showing a decrease in the blood sugar levels may be due to the stimulation of residual pancreatic mechanism or by increasing peripheral utilization of glucose[5]. It is also effectively used for the diabetes related symptoms such as hyperlipidemia and hyperglycemia in the Indian sub-continent[22]. Several reports revealed that the seed extracts of *M. peregrina* is used as potent antidiabetic agent[23]. Against the rested diabetic mice, the n-hexane seed extract (-101.4 mg/dL) of *M. peregrina* showed more anti-diabetic activity in reducing blood sugar levels than the leaf extract (-83.7 mg/dL) than standard drug metformin treated group (-88.9 mg/dL). The findings of blood sugar reduction by *M. peregrina* seed and leaf extracts may be either through gluconeogenesis or by pancreatic changes which leads the pancreatic cells to become normal and allowing blood sugar to enter into cells. The hypoglycemic and other related properties of *M. peregrina* is due to the presence of rich bioactive principles among the plant diversity[5]. Some reports have suggested that saponins and flavonoids as a secondary metabolites present in the plants are widely responsible for the antidiabetic activity[24-25].

In relation to diabetes and its relation with body weight gain or loss, the study was conducted to determine the effect of *M. peregrina* on alloxan-induced diabetic mice. The results showed that the plant extracts studied were effective in maintaining the body weight without any significant changes. The seed extract of *M. peregrina* showed a positive result in the increase of the body weight of diabetic mice (+0.6 g) similarly to the metformin treated mice (+2.6 g). While the leaf extract treated mice showed negative results in decreasing body weight in diabetic mice (-1.4 g), similar to the untreated diabetic mice (-0.3 g). The weight gain or loss in the diabetic mice may be dependent on dosage, physiology of the mice metabolic rate, and various other internal factors. In general type I diabetes is directly proportional to weight gain. In type II diabetes, due to the unavailability of blood glucose for the cells, the muscle reserves starts to burn and leads to weight loss. However, if diabetics are under treatment and the drug is effective in controlling the sugar levels then the body weight will be managed to normal levels. The results are shown in Table 2. It is observed

that the diabetic mice treated with Metformin as well seed extracts of *M. peregrina* showed positive results when compared with untreated diabetic mice.

Conclusion

The seed and leaf extract of *M. peregrina* have positive effect as antihyperglycemic agent on diabetic mice while the seed extract showed a significant reduction of blood sugar levels in diabetic mice also it has a positive effect on body weight, reduced the blood sugar level more than leaf extract. More detailed study of these plant materials is required to establish conditions for pharmacological preparations that may be useful in treating diabetes.

Conflict of interest

The authors declare that there is no conflict of interests to publish these results.

References

1 Wild S H, Roglić G, Green A , Sicree R A and King H, Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030, *Diabetes care*, 2004, **27**(5), 1047-1053.

2 Levin M and Pfeifer M, *The uncomplicated guide to diabetes complications*, (American Diabetes Association, Alexandria, Virginia); 2009.

3 Ivorra M D, Payá M and Villar A, A review of natural products and plants as potential antidiabetic drugs, *J Ethnopharmacol*, 1989, **27**(3), 243-275.

4 Gad M Z, El-Sawalhi M M, Ismail M F and El-Tanbouly N D, Biochemical study of the anti-diabetic action of the Egyptian plants fenugreek and balanites, *Mol Cell Biochem*, 2006, **281**(1-2), 173-183.

5 Reddy S H, Al-Neeri I S, Al-Issaei H K and Al-Jabri S A, Effect of selective medicinal plant extract on blood glucose, sperm shape and various physiological parameters, *Am J Plant Sci*, 2015, **6**, 1109–1115.

6 Singh S K, Rai P K, Mehta S, Singh R K and Watal G, Curative effect of *Cynodondactylon* against STZ induced hepatic injury in diabetic rats, *Indian J Clin Biochem*, 2009, **24**(4), 410-413.

7 Nadro M S, Audu A S and Glen E, Anti-diabetic effects of aqueous extract and oil of *Moringa oleifera* seed on liver and kidney functions in Streptozotocin-induced diabetes in Rats, *Am J Biochem*, 2018, **8**(4), 69-74.

8 Marwah R G, Fatope M O, Al Mahrooqi R, Varma G B, Al Abadi H, *et al.*, Antioxidant capacity of some edible and wound healing plants in Oman, *Food Chem*, 2007, **101**, 465–470.

9 Abd El-Wahab R, *Reproduction ecology of wild trees and shrubs in southern sinai, Egypt*, Master thesis, Botany Department, Faculty of Science, Suez Canal University, Ismailia, 1995.

10 Robiansyah I, Hajar A S, Al-kordy M A and Ramadan A, Current status of economically important plant *Moringa peregrina* (Forrsk.) Fiori in Saudi Arabia, a review, *Int J Theor Appl Sci*, 2014, **6**, 79–86.

11 Olson M E, Sankaran R P, Fahey J W, Grusak M A and Odee D, Leaf protein and mineral concentrations across the "Miracle Tree" genus *Moringa*, *PloS one*, 2016, **11**(7), e0159782.

12 Tsaknis J, Characterisation of *Moringa peregrina* Arabia seed oil, *Grasas Y Aceites*, 1998, **49**(2), 170-76.

13 Somali M A, Bajnedi M A and Al-Faimani S S, Chemical composition and characteristics of *M. peregrina* seeds and seed oil, *J Am Chem Soc*, 1984, **61**, 85-86.

14 Senthilkumar A, Karuvantevida N, Rastrelli L, Kurup S S and Cheruth A J, Traditional uses, pharmacological efficacy, and phytochemistry of *Moringa peregrina* (Forssk.) Fiori. - A review, *Front Pharmacol*, 2018, **9**, 465.

15 Ghazanfar S A, *Handbook of Arabian Medicinal Plants*, (CRC Press, London), 1994, 144.

16 Salla H R, Al Habsi F S, Al dholi H M, Al musallami S T and Al Sharji W H, A comparative study on the role of Omani honey with various food supplements on diabetes and wound healing, *J King Saud Univ - Sci*, 2020, **32**(3), 2122-2128.

17 Hemadri Reddy S, Al-Rawahi A S and Al-Kalbani A S, Hypoglycemic effect of black cumin (*nigella sativa*) seed and Senna alexandria (*Cassia angustifolia*) leaf extracts on alloxan-induced mice, *J Herbs Spices Med Plants*, 2017, **23**(4), 401–408.

18 Collin C, Davies P, Mutiboko I K and Ratcliffe S, Sativex spasticity in MS Study Group, Randomized controlled trial of cannabis based medicine in spasticity caused by multiple sclerosis, *Eur J Neurol*, 2007, **14**(3), 290-296.

19 Ifesan B O T, Fashakin J F, Ebosele F and Oyerinde A S, Antioxidant and antimicrobial properties of selected plant leaves, *Eur J Med Plants*, 2013, **3**(3), 465-473.

20 El-Alfy T S, Ezzat S M, Hegazy A K, Amer A M and Kamel G M, Isolation of biologically active constituents from *Moringa peregrina* (Forssk.) Fiori. (family: Moringaceae) growing in Egypt, *Pharmacogn mag*, 2011, **7**(26), 109.

21 El-Haddad A E, Koheil M A, El-Khalik S M A and Osman S, Antihyperglycemic activity and nitrile glycosides of *Moringa peregrina* (Forssk.) seeds, in *Fourth Euro-Mediterranean Conference of Natural Products and Drug Discovery: Back to Mother Nature*, (Cairo/Sharm El-Sheikh: BioNat-IV), 2002.

22 Koheil M A, Hussein M A, Othman S M and El-Haddad A, *In-vivo* antioxidant activity of *Moringa peregrina* against STZ– induced oxidative stress in type 2 diabetic rats, *Mol Clin Pharmacol*, 2013, **4**, 65–75.

23 Hemadri Reddy S, Al-Rawahi A S and Al-Kalbani A S, Hypoglycemic effect of black cumin (*nigella sativa*) seed and Senna alexandria (*Cassia angustifolia*) leaf extracts on alloxan-induced mice, *J Herbs Spices Med Plants*, 2017, **23**(4), 401–408.

24 Najafian M A, Ebrahim-Habibi P, Yaghmaei K P and Larijani B, Core structure of flavonoids precursor as an anti-hyperglycemic and anti-hyperlipidemic agent: An *In vivo* study in rats, *Acta Biochim Pol*, 2010, **57**, 553-560.

25 Elekofehintia O O, Kamdem J P, Kade I J, Rocha J B T and Adanlawod I G, Hypoglycemic, antiperoxidative and antihyperlipidemic effects of saponins from *Solanum anguivi* Lam. fruits in alloxan-induced diabetic rats, *S Afr J Bot*, 2013, **88**, 56-61.