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Influence of Fatty Acid Profile of *Moringa peregrina* Seeds Oil on Blood Plasma Lipids and Blood Glucose on Rats

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ABSTRACT

Background: Residents of Saudi Arabia believe that *Moringa peregrina* seeds oil has a positive health effect on blood cholesterol and blood glucose. In this study, *Moringa peregrina* seeds oil (MPO) was compared with corn oil on plasma lipids, blood glucose and adipose tissues to study their influence on plasma parameters and obesity.

Methods: Fifteen male albino rats were divided into three groups, each group containing five rats. The control group consumed a normal diet, the MPO group consumed a normal diet and 20% Moringa oil and the CO group consumed a normal diet and 20% corn oil for 8 weeks. The data were analyzed by one-way ANOVA with ($p<0.05$).

Result: According to results, no significant differences between MPO and CO on the plasma lipids. While there are no significant differences in blood glucose levels between the control group 75.36±8.05 mg/dl and the MPO group 90.62±0.88 mg/dl and there is a significant increase in the CO group 107.28±3.90 mg/dl. No significant differences between all groups in BMI and final weight. While, the highest total percentage of adipose tissues was in the MPO group compared to CO, which was 1.72±0.13 in the MPO group and 1.36±0.12 in the CO group. *Moringa peregrina* seed oil does not lower blood glucose levels as it is a popular belief but does reduce the blood glucose spikes compared to corn oil. Additionally, substituting it for corn oil does not alter plasma lipid levels and it increases obesity factors (adipose tissues).

Key words: Adipose tissues, Blood glucose, Corn oil, *Moringa peregrina*, Plasma lipids.

INTRODUCTION

Moringa peregrina seeds oil has a cultural and religious connection with the inhabitants of the Arabian Peninsula in terms of the medicinal effects of moringa, such as high blood pressure, diabetes, anti-inflammatories and lowering blood lipids (Senthilkumar *et al.,* 2018). Additionally, in the Kingdom of Saudi Arabia, some believe that moringa peregrina oil has a medicinal effect on blood sugar and lipids (triglyceride, total cholesterol, high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) levels). Moringa Peregrina seeds contain 2.4% moisture, 53.5% fat, 24.1% protein and 2.6% ash. The seeds oil contains 83.5% unsaturated fatty acids, oleic acid as a mean fatty acid 74.8%, palmitic acid 8.9%, stearic acid 3.1% and behenic acid 2.6% (Al-Dabbas *et al.,* 2010).

In an experimental study, they found that Moringa peregrina seeds extract has a potential anti-obesity effect in rats as a supplement in a high-fat diet (Elarabany *et al.,* 2022). A study showed that the Albino rats who consumed 500 mg/kg of dry seed of Moringa peregrina had a significant decrease in blood glucose compared with rats who consumed 0, 1000 and 2000 mg/kg of dry M. peregrina seed (EL-Hak *et al.,* 2018). However, a review study concluded that there are no enough evidences on moringa that is strong enough to recommend it as a treatment for diabetes (Owens *et al.,* 2020). A study assessed the impact of *Moringa peregrina* seed oil on obesity in rats fed a high-fat diet. The consumption of this oil notably improved body weight gain and counteracted the diet-induced increases in cholesterol, triglycerides and LDL-C, while also reducing HDL-C levels. Additionally, *Moringa peregrina* seed oil demonstrated strong free radical-scavenging activity (Elarabany *et al.,* 2022). In a study, the effect of *Moringa peregrina* on lowering blood lipids in rats with hyperlipidemia was investigated. The study concluded that Moringa extract had a better effect than hyperlipidemia medications (Aborhyem *et al.,* 2016). The effect of *Moringa peregrina* on blood lipids was studied in healthy individuals for 14 days and the study concluded that there was no statistically significant effect on blood lipids or significant reduction in blood pressure or body mass index when consuming Moringa as a dietary supplement compared to those who did not consume it (Seriki *et al.,* 2015).

Corn oil, rich in unsaturated fatty acids, has been shown in numerous studies to positively affect plasma lipid levels compared to other oils and fats (Kevin Maki

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et al., 2018; Maki *et al.,* 2017). A comparison study of the effects of corn oil and extra virgin olive oil on blood lipids recommended that corn oil positively affects low-density lipoproteins and lipids associated with atherosclerosis (Kevin Maki *et al.,* 2015). Corn oil and coconut oil have the same effect in lowering blood glucose in Wistar rats injected with alloxane (Firdausi *et al.,* 2017). Rats fed corn oil showed lower levels of blood glucose, triglycerides and LDL-C than rats fed beef tallow or different types of palm oil and the corn oil group had the highest levels of HDL-C (Utama *et al.,* 2024).

Blood glucose, triglycerides, ALT and AST were not affected by consuming 125 ml or 250 ml of sunflower oil for 90 days, but sunflower oil intake led to a significant increase in blood cholesterol compared to the control group (Ahuja *et al.,* 2023). Rats fed black seed oil and beef tallow had significantly lower weight gain, lower levels of triglycerides, total cholesterol and LDL-C and higher levels of HDL-C compared to rats fed beef tallow alone (Miah *et al.,* 2023). Feeding heifers on palm oil, soybean oil or linseed oil did not affect blood glucose, triglycerides and LDL-C while total cholesterol and HDL-C were higher in the soybean oil group or linseed oil group compared to the palm oil group (Kumar *et al.,* 2022).

Many studies studied Moringa Peregrina seeds oil effect as a nutritional or therapeutic supplement. In this study, the effect of Moringa Peregrina seeds oil on blood lipids, blood glucose and obesity will be identified compared to another type of oil, which is corn oil, in a high-fat diet. The differences in these indicators mentioned between the two types of oil will be identified to determine which of them has the best positive effect on blood glucose, blood lipids and obesity.

MATERIALS AND METHODS

Moringa Peregrina seeds oil was purchased from a local farm in the north region of Saudi Arabia. Corn oil was obtained from a local market. Animal feed suitable for rats was purchased from a local market (Tunai brand). Plasma kits for blood glucose level, total cholesterol level, HDL-C level and total triglyceride level were obtained from QCA, Spain.

Composition of fatty acids profile

Gas chromatography (GC) was used for the composition of Fatty acids profile by the AOAC-996.01 method (Satchithanandam *et al.,* 2001). By using GC-2010 Plus (Shimadzu, Japan) with split injection mode and the carrier gas was Helium gas and column Fused silica 100 m* 0.25*0.2 µm and the detector was flam ionization. The separation temperature was 100°C and the separation time was 71 min.

The biological experiment

The Research Ethics Committee in the Deanship of Scientific Research-King Faisal University, Al-Ahsa, Saudi Arabia, approved the experiment with reference number KFU-REC-2021-NOV-EA000164. The experiment was done in the Department of Food and Nutrition Sciences, College of Agricultural and Food Sciences, King Faisal University, Al-Ahsa, Saudi Arabia. The experiment was conducted in December 2023 for a period of four months.

Fifteen male Albino rats were purchased from the animal house weighing between 80 to 100 grams and their age was about four weeks. They were divided into three groups and kept in the normal temperature and humidity (25°C, 40%), with 12 hours of light and 12 hours of dark for the period of the experiment. After one week of accumulation, the rats started to feed on the experimental diet, which was three different diets. The first group received a normal diet (control), the second group received a high-fat diet consisting of the normal diet plus 20% corn oil (CO) and the third group was given a high-fat diet made up of the normal diet plus 20% Moringa peregrina seed oil (MPO) Table (1). Rats were coded in their tail for continuous follow-up. All groups were fed on their diets for eight weeks with an open quantity of food and water. The food intake was recorded for the groups. Body weight from the zero time and week four and at the end of the experiment was taken for all rats to determine the percentage of body weight gain (%BWG) and body mass index (BMI). From the zero time (before starting feeding on the different diets), blood was collected from all rats and at the end of week eight. After the eighth week, all rats were dissected to obtain and weigh some organs such as the heart, liver, kidneys, spleen and white adipose tissue. The liver was kept in a 0.9% saline solution to perform an analysis of cholesterol and triglycerides in the liver.

Blood plasma collection

Blood samples were collected from the eye vein according to (Abd El Malik, 2019). Sodium heparinized capillary tubes were used to collect 1.5 ml of blood from each rat in an Eppendorf tube containing EDTA. Then, the tubes were centrifuged at 3000 rpm for 18 min. The plasma from each tube was transferred to a new Eppendorf tube to obtain the blood glucose, total triglycerides (TG), total cholesterol (TC) and high-density lipoprotein (HDL-C).

Plasma lipids and blood glucose

Blood glucose level, total triglycerides level, total cholesterol level and HDL-C level were analyzed as described in the kit manufacturer’s instructions using a spectrophotometer UV-Vis. LDL-C and VLDL-C were calculated as ″LDL and VLDL-C = TC – HDL-C″ as described by (Reis *et al.,* 2021).

Liver TG and TC

Liver TG and TC were determined as (Montano *et al.,* 1998) described. The liver from each rat was mashed gently, then

Table 1: Diet composition (g/100 g).

Group/Ingredient	Normal rat food	MPO	CO
Control group	100%	0%	0%
MPO group	80%	20%	0%
CO group	80%	0%	20%

Moringa peregrina seeds oil (MPO). Corn oil (CO).

weighed 0.1 g in a stoppered test tube, then adding 10 ml from a mixture of chloroform and methanol 2:1, then shaking the test tube very well, then filtering the test tube content. 10 microliters from the filtered solution were taken in a clean test tube, adding 1 ml of TG or TC reagent and incubating the solution at room temperature (20-25 C) for 10 min, then the absorbance was at 546 nm. A standard sample was taken in the same procedure.

$$\text{Liver TG mg/g} = \frac{\text{Sample Abs}}{\text{Standard Abs}} * 200$$

$$\text{Liver TC mg/g} = \frac{\text{Sample Abs}}{\text{Standard Abs}} * 200$$

Body mass index (BMI) and % body weight gain (%BWG)

BMI and %BWG were calculated as (Abd El-Gawad *et al.,* 2005) describe:

$$\text{Body mass index} = \frac{\text{Final weight by gram}}{\text{Length}^2 \text{ by cm}}$$

$$\text{\% Body weight gain} = \frac{\text{Final weight - Initial weight}}{\text{Initial weight}} * 100$$

Statistical analysis

The statistical analysis was done by using SAS09 software. All the results were presented as mean ± STD and means values were tested using least significant differences (LSD) with a significant level ($p<0.05$).

RESULTS AND DISCUSSION

Table (2) promotes the fatty acids composition in *Moringa peregrina* seeds oil and corn oil. The predominant fatty acids type in both oils are unsaturated fatty acid by 81.58% in MPO and 85.98% in corn oil. MPO has a large amount of MUFA compared to CO and on the other hand, CO has a high proportion of PUFA (57.24%) and MPO has (1.1%). In addition, the saturated fatty acids in MPO and CO were 18.42% and 14.02% respectively. The trans fatty acids are undetected in corn oil, but there are a small amount in MPO (0.09%). However, the predominant fatty acids in MPO were oleic acid 76.93%, palmitic acid 9.64% and stearic acid 4.02%. While the main fatty acids in corn oil were linoleic 57.18%, oleic acid 28.63% and palmitic acid 11.57%. These results agreed with other studies results, the predominant fatty acid in Moringa peregrina oil seeds is oleic acid 70.52% and palmitic acid 8.90% (Tsaknis, 1998). Instead, corn oil contains 59% PUFA primarily linoleic acid and 24% monounsaturated fatty acids (Dupont *et al.,* 1990).

Fig 1 illustrate the food consumption in different groups at week 1 and week8. No significant differences between groups in the quantity of food intake at the first week and the eighth week.

Table (3) demonstrates the plasma lipids and blood glucose in rats at the beginning time and after eight weeks of feeding on different diets. According to Harini and Astirin total cholesterol level of the plasma in white rats is between 10 and 54 mg/dl (HARINI *et al.,* 1970). However, all groups

Table 2: Fatty acids composition of moringa peregrina seeds oil and corn oil.

Fatty acids (%)	MPO	CO
Myristic C14:0	0.08	0.04
Pentadecanoic C15:0	Not detected	Not detected
Palmitic C16:0	9.64	11.57
Palmitoleic C16:1	2.07	0.1
Heptdecanoic C17:0	0.06	0.08
CIS-10 Heptadecanoic C17:1	0.04	0.01
Stearic C18:0	4.02	1.7
Elaidic C18:1n9t	0.09	Not detected
Oleic C18:1n9c	76.93	28.63
Linoleic C18:2n6c	1.03	57.18
Arachidic C20:0	1.71	0.37
Cis-11-Eicosenoic C20:1n9	1.33	Not detected
A-Linolenic C18:3n3	0.05	0.06
Henecosanoic C21:0	0.05	ND
Behenic C22:0	2.42	0.1
Erucic C22:1n9	0.02	Not detected
Cis-11,14,17-Eicosatrienoic C20:3n3	0.02	Not detected
Lignoceric C24:0	0.44	0.16
Saturated fatty acid (SFA)	18.42	14.02
Unsaturated fatty acid (UFA)	81.58	85.98
Monounsaturated fatty acid (MUFA)	80.48	28.74
Polyunsaturated fatty acid (PUFA)	1.1	57.24
Trans fatty acid (TFA)	0.09	Not detected

in this study were in the normal range. The highest TC levels were found in the MPO group with no significant differences compared to the corn oil group, while the lowest TC level was found in control group.

HDL-C levels between 21-54 mg/dl are classified as normal levels in white rats reported by (Abd El Malik, 2019). However, all treatments are within the range of normal HDL-C levels. In our study, the MPO group recorded the highest HDL-C with no significant differences compared to the corn oil group and the lowest levels of HDL-C were found in the control rats.

The LDL-C and VLDL-C levels in all groups start from 4.01 to 11.88 mg/dl. The lowest LDL-C and VLDL-C levels were found in the normal diet group (control) and this may be due to their balanced diet in nutrient components. However, no significant differences were observed between the corn oil group and the MPO group on LDL-C and VLDL-C levels.

The normal range of triglyceride levels in the rat plasma is between 26 and 145 mg/dl (Mahdi *et al.,* 2020). According to our results, the TG levels in all groups were from 32.31 to 39.20 mg/dl. The control group had the lowest TG levels, showing no significant difference with the MPO group but a significant difference compared to the corn oil group. However, there is no significant effect on TG when rats consume Moringa Peregrina seeds oil compared to rats consuming corn oil for 8 weeks. A study investigated the impact of corn oil, which is rich in polyunsaturated fatty acids, on blood lipids and compared it to an oil high in monounsaturated fatty acids (olive oil). The study concluded that corn oil had a more favorable impact on plasma lipids compared to olive oil (Wagner *et al.,* 2001).

In this study, we found that rats consuming a diet containing more oil could affect their blood glucose levels. According to the results illustrated in Table (3), the lowest blood glucose levels were found in the control group with significant differences compared to other groups. However, all blood glucose levels of rats were in the range of normal blood glucose in white rats, which is from 50 to 135 mg/dl (Hidayaturrahmah *et al.,* 2020). Blood glucose levels were significantly higher in the corn oil group compared to both the MPO and the control groups. *Moringa peregrina* seeds oil did not lower blood glucose levels directly, it has been observed reduce the glucose spikes. Compared to corn oil, Moringa peregrina oil may offer a more stable blood glucose level. *Moringa peregrina* seeds oil had a slight increase in blood glucose level compared to the control group and this may be due to this oil having more monounsaturated fatty acids. Monounsaturated fatty acids could affect glucose levels by reducing insulin resistance. Low-density lipoprotein oxidation may have an effect on increasing insulin sensitivity. Oxidized LDL has been associated with impairment in vascular reactivity (Ryan *et al.,* 2000). Consuming a diet rich in oleic acid reduces the level of low-density lipoproteins (Karacor *et al.,* 2015). This effect of MPO can be beneficial in managing blood glucose levels, especially in individuals at risk of diabetes or those with glucose metabolism disorders.

Table 4 promotes %BWG, origins weight, adipose tissues and liver lipids. According to table (IV), there are no significant differences in liver TG between all groups, but liver TC was lower in the control group compared to other groups. No significant differences in liver TC were found between corn oil and MPO groups.

Liver TG level in the MPO group is 23.48 mg/g and 26.71 mg/g in the CO group. And these differences agreed with a study studying different types of oil and fat on liver TG. They found olive oil which rich in oleic acid lower in TG lever compared to corn oil (Gao *et al.,* 2019). A diet high in oleic acid lowers the hepatic levels of α-linolenic acid (ALA) and eicosapentaenoic acid (EPA), while simultaneously increasing the expression of the genes responsible for Δ5 and Δ6 desaturases (Picklo *et al.,* 2016).

The normal range for BMI in Wistar rats is from 0.45 to 0.68 g/cm^2 (Novelli *et al.,* 2007). No significant differences

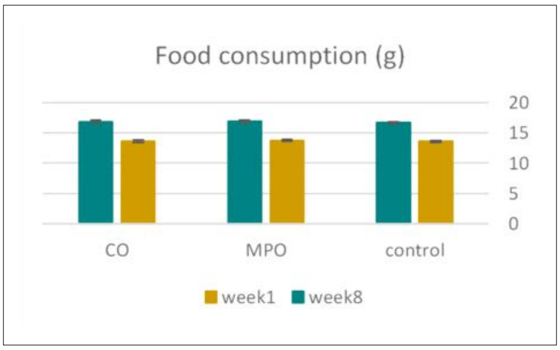

Fig 1: Food intake for different groups in week1 and week 8.

Table 3: Effect of fed on different types of oil on plasma parameters.

Parameter	Group			
	Beginning	Normal	Corn oil	*Moringa peregrina* oil
TC mg/dl	36.50±7.54	27.12±2.25^b	47.39±4.31^a	49.07±9.36^a
HDL mg/dl	25.52±7.07	23.11±1.78^b	35.51±3.24^{ab}	37.77±5.78^a
LDLandVLDL mg/dl	10.98±8.02	4.01±2.02^b	11.88±1.79^a	11.29±5.12^a
TG mg/dl	32.25±10.94	32.31±5.18^b	39.20±4.73^a	34.17±1.96^{ab}
Glucose mg/dl	70.71±8.65	75.36±8.05^c	107.28±3.90^a	90.62±0.88^b

Data are shown as mean±SD on n=5 replicate per group. Values with different latter are significant different, p<0.05. ab= No significant different with data having a or b.

Table 4: Effect of fed on different types of oil on body weight, BMI, %BWG, origins weight, adipose tissues and liver lipids.

	Group		
Parameter	Normal	Corn oil	Moringa peregrina oil
Initial weight g	119.6±20.13[a]	116.6±12.30[a]	131.6±25.80[a]
Final weight g	161.6±35.18[a]	213.2±37.81[a]	211.2±56.85[a]
BMI g/cm^2	0.46±0.09[a]	0.47±0.03[a]	0.50±0.02[a]
%BWG	34.59±13.38[b]	83.48±31.70[a]	59.77±26.40[ab]
% Heart	0.47±0.03[a]	0.34±0.01[b]	0.34±0.02[b]
% Liver	4.25±0.03[ab]	4.42±0.22[a]	4.13±0.27[b]
% Kidney	0.90±0.12[a]	0.76±0.06[b]	0.69±0.01[b]
% spleen	0.64±0.10[a]	0.30±0.03[b]	0.34±0.08[b]
% Total adipose tissues	0.99±0.02[c]	1.36±0.12[b]	1.72±0.13[a]
% epididymal	0.25±0.02[c]	0.42±0.04[b]	0.56±0.07[a]
% mesenteric	0.64±0.02[b]	0.59±0.09[b]	0.77±0.05[a]
% retroperitoneal	0.04±0.01[c]	0.25±0.02[b]	0.32±0.04[a]
% perirenal	0.04±0.006[c]	0.09±0.006[a]	0.06±0.007[b]
Liver TC mg/g	1.10±0.08[b]	2.76±0.55[a]	3.54±0.93[a]
Liver TG mg/g	26.94±5.18[a]	26.71±8.80[a]	23.48±2.72[a]

Data are shown as mean±SD on n=5 replicate per group. Values with different latter are significant different, $p<0.05$. ab= No significant different with data having a or b.

were found in BMI between groups. However, the body mass index (BMI) is inaccurate and poor as obesity index in some cases in some human populations or animals and the percentage of body fat is an accurate index for obesity (Rodríguez-Correa *et al.,* 2020). The lowest %BWG was in control rats with no significant differences compared to MPO and a significant difference with the corn oil group.

Table (IV) demonstrates different adipose tissues were collected from rats as a percentage. The highest proportion of total adipose tissues, epididymal, mesenteric and retroperitoneal was in the MPO group with significant differences compared to other groups and the lowest proportions were in control rats. The percentage of perirenal in the corn oil group was more than the MPO group and it is significant.

CONCLUSION

Based on the results of this study, it can be concluded that Moringa peregrina seeds oil reduces the spikes of blood glucose levels. Compared to corn oil, *Moringa peregrina* oil may offer a more stable blood glucose level. The fatty acids composition of MPO may influence the blood glucose as it is high of monounsaturated fatty acids. Furthermore, there are no substantive changes in plasma lipids when consuming corn oil or M. peregrina seeds oil. Consuming a high-fat diet may cause an increase in liver cholesterol, while this diet did not significantly affect liver triglycerides. Moringa pergrina seeds oil could also reduce the ratio of body weight gain in a high fat diet compared to corn oil. Corn oil may have a positive effect on white adipose tissues, which are an accurate indicator for diagnosing obesity in humans or animals. Conduct more studies on Moringa pergrina seeds oil and the unsaponifiable matters in the oil and link their health effects to various health indicators, which are under this study. In addition, conduct studies on the oils used in this study needed to reach a suitable mixture between Moringa pergrina seeds oil and corn oil to achieve balance in the health indicators under this study.

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Authors' contribution

Shaheed M Alshaikhsaleh in lab analysis included the biological experiment and blood samples analysis, literature review and editing the article. Saeed A Asiri in lab analysis including chemical analysis and reviewing the article.

Data availability

Data will be available on request to the corresponding author.

Ethics approval statement

The experiment was approved by the Research Ethics Committee in the Deanship of Scientific Research – King Faisal University, Saudi Arabia, KFU-REC-2021-NOV-EA000164.

Conflict of Interest

The authors declare no conflict of interest.

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