The Use of Maca (Lepidium meyenii) to Improve Semen Quality in Diabetic Obese Male Rats

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Abstract

The effect of maca root extract on improving sperm status in obese diabetic male rats was studied. Thirty-five rats were used and divided into five groups: a healthy positive group, four groups with obesity and diabetes, a negative group, and three groups fed maca root extract at levels of 100, 300, and 500 ml for six weeks. The rats were slaughtered, serum was extracted, and the following estimates were made: Blood lipid profile (TC), total cholesterol (TG), triglycerides, very low-density cholesterol (VLDL-C), high-density cholesterol (HDL-C), low-density cholesterol (LDL-C), liver functions (AST, ALT, ALP), sex hormones (Testosterone, FSH, LH), antioxidants (CAT, MDA), sperm ratio, and glucose. The results showed a significant improvement in liver functions, blood lipids (triglycerides, total cholesterol), a decrease in harmful cholesterol, and an increase in good cholesterol. Improve in Sex hormones and sperm percentage in rats fed maca root extract compared to negative groups.

Key words

Maca , Semen , Diabetic , Obese , Rat

Introduction

According to the WHO, around 20% of developed-country couples and 25% of Un developing-country couples have experienced infertility at some point in their relationship. According to epidemiological studies, there may be up to 186 million infertile couples worldwide. It is a problem that affects between 15% and 20% of marriages, according to experts **(WHO, 2020).** The prevalence of primary and secondary infertility in Egypt was 9 and 16%, respectively **(Sanad et al., 2019).** It is recorded that an estimated 35% of infertility cases include only women, 20% both women and men, 30% include problems only on the

part of the man, and 15% of infertility cases remain unexplained **(Leaver, 2016).**

Maca (Lepidium meyenii) belongs to the Brassicaceae family and the Lepidium genus has several possible uses include improving sexual function, and fertility by impact of hormones levels specifically Luteinizing Hormone (LH) concentrations. the improvement of sperm function, nevertheless the level of evidence is limited. This suggests the effectiveness of Maca but more systematic clinical studies are needed **(Sánchez et al., 2017)**. Maca root is an adaptogenic herb that has been shown in studies to improve fertility in both men and women. It contains about 31 different minerals and 60 different phytonutrients. Maca can affect key hormones in both women and men without containing hormones itself (**Hethir, 2007**). Black Maca increased daily sperm production and efficiency, suggesting that Black Maca may become a potential treatment for male infertility **(Gonzalez et al., 2006)**. Studies suggest that maca regulates sperm count by maintaining the balance between oxidant and antioxidant status (**Yucra et al., 2008)**. Moreover, the benefits of maca have been demonstrated in several conditions such as prostatic benign hyperplasia, osteoporosis in adults' rats and reduce glycaemia in diabetes mellitus **(Gonzales-Arimborgo et al., 2016**). The prevalence of obesity and diabetes mellitus (DM) has been consistently increasing worldwide. Obesity has become a global pandemic threatening people's life by affecting almost every organ system and is now a severe public health problem as one of the most common non-communicable diseases (NCDs) **(Blüher, 2019 and Tsai and Bessesen, 2019)**. Sharing powerful genetic and environmental features in their pathogenesis, obesity amplifies the impact of genetic susceptibility and environmental factors on DM. The ectopic expansion of adipose tissue and excessive accumulation of certain nutrients and metabolites sabotage the metabolic balance via insulin resistance, dysfunctional autophagy, and microbiome-gut-brain axis, further exacerbating the dysregulation of immunometabolism through low-grade systemic inflammation, leading to an accelerated loss of functional β-cells and gradual elevation of blood glucose. Given these intricate connections, most available treatments of obesity and type 2 DM (T2DM) have a mutual effect on each other **(Ruze *et al.*, 2023).** This study was conducted to evaluate the effectiveness of black maca on semen quality and sex hormones concentrations in obese diabetic rats.

Materiel and methods

Materials:-

Chemicals: Casein, vitamins, minerals and cellulose were purchased from El-Gomhoria Company – Cairo – Egypt.

Herbs: Maca (Lepidium meyenii) roots was obtained from the local market for Herbs and Medicinal Plants, Cairo, Egypt.

Animals: Adult male albino rats (Sprague- Dawley strain) (n=35 rat) weighing approximately (180 ± 5 g.) were purchased from Helwan Experimental Animals Station.

Kits: Kits for blood analysis were purchased from Gama Trade Company for Chemical, Dokki, Egypt.

Methods:

1- The scientific identification of Maca (*Lepidium meyenii*) roots was carried out at the Agriculture Research Center.

2- Preparation of the alcoholic root extract:

Maca **roots** was grinded then extracted 50 g of the plant roots using 200 mL of 70% ethanol at room temperature, macerated for 72 hours, then filtered three times and evaporated to obtain a crude Maca extract**.**

3- Induction of Diabetic rats: Diabetes was induced by a single intraperitoneal injection of freshly prepared STZ (60 mg/kg BW). After Three days, random blood samples were taken then the level of the blood glucose was assessed and the level ≥250 mg/dl was considered as diabetic **(Sarkar et al., 1996).**

4- Induction of obesity model : Rats were fed four weeks on basal diet according to **(Reeves, et al., 1993)** with some modification in fat content (HFD) for four weeks and the remainder was starch to induce obesity in rats **(Liu, et al., 2004).**

5- Biological study:

The basal diet will be formulated according to **Reeves, et al., (1993).** After adaptation period, thirty-five rats were divided into two main groups as follows:-

❖ **the first main group** (7 rats) was fed on basal diet (as a control negative group).

❖ **The second main group** (28 diabetic obese rat) were divided into 4 subgroups (7 rats each):

➢ subgroup (1) fed on basal diet (as a control positive group),

➢ subgroup (2) fed on basal diet and given orally (100mg Extract of maca root /kg BW),

➢ subgroup (3) fed on basal diet and given orally 300mg Extract maca root /kg BW),

➢ subgroup (4) fed on basal diet and given orally (500mg Extract maca root /kg BW).

6-Nutritional evaluation: The biological evaluation of the diet was carried out by determination of feed intake, body weight gain percent and feed efficiency ratio.

At the end of the **experimental period (6 weeks),** rats were fasted overnight before sacrificing, dissected under slight anesthesia by ether and the blood samples were collected from each rat and will be centrifuged to obtain serum which stored at -20°C until biochemical analysis. Testis were removed, weighed then used for determination semen quality.

7-Biochemical Analysis:

1-Serum will be used to determine the following parameters :

- **Hormones**: Follicular stimulating hormone (FSH) , luteinizing hormone (LH) and Testosterone hormone
- **Liver functions** (Aspartate and alanine aminotransferase activities, Alkaline phosphatase).
- Malondialdehyde and catalase
- **Lipid Profile:** Total cholesterol, Serum triglyceride, High density lipoprotein–cholesterol, Low density lipoprotein–cholesterol and Very Low-density lipoprotein–cholesterol.
- **Glucose**

2. **Semen Analysis:** Semen analysis was determined

3. <u>Biological study:</u>

• Preparation of Basal Diet:

The basal diet was formulated according to **A.O.A.C (1995)** and presented in **Table (c)**

Table (a): Composition of the basal diet

Ingredients	**g %**
Corn starch	67.2
Casine	15
Methionine	0.3
Oil	7.5
Mineral mixture	4
Vitamin mixture	1
Cellulose	5

Table (b): Vitamin mixture and mineral mixture

Mineral mixture ingredients	G		Vitamin mixture ingredients	g
NaCl	139.3		**Menadione**	0.5
KI	0.79		**p-Aminobenzoic acid**	10
KH_2PO_4	389		**Inositol**	10
$Mg\,SO_4$	57.3		**Niacin**	4
$CaCO_3$	381.4		**Ca D-pantothente**	4
$FeSO_4.7H_2O$	27		**Riboflavin**	0.8
$MnSO_4.\,H_2O$	4.01		**Thamine-Hcl**	0.5
ZnSO4.7H2O	0.548		**Pyridoxine-Hcl**	0.5
CuSO4.5H2O	0.477		**Folic acid**	0.2
$CoCl_2$	0.023		**Biotin**	0.04
			Vitamin B_{12}	0.003
			Glucose, to make	1000

Mineral mixture and vitamin mixture were prepared according to **A.O.A.C, (1995)**. Cod liver oil (5ml /kg diet) was added to supply 2000 IU vitamin A and 200 IU vitamin D. Choline chloride (5 ml of 20% solution / kg diet) was added at the time of preparation.

Result and discussion

Table **(1): Effect of maca root extract on body weight in diabetic obese male rats**

Parameters / Groups	IBW (g)	FBW(g)	BWG%	FI (g/d/rat)	FER
Control (-ve)	177.33±0.85a	200.56±0.90d	13.10±0.39d	16.50	0.050±0.014d
Control (+ve)	175.76±1.59a	231.50±1.42a	31.72±0.62a	20.50	0.097±0.013a
mg Extract of 100 maca root /kg BW	178.60±1.08a	223.06±1.06b	24.91±1.11b	19.00	0.084±0.033b
300 mg Extract of maca root /kg BW	178.23±1.22a	220.43±1.17b	23.68±0.51b	17.50	0.086±0.016b
500 mg Extract of maca root /kg BW	178.30±1.83a	207.03±0.51c	16.16±1.46c	17.20	0.060±0.048c

Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at ($P<0.05$).

Table (1) showed the Effect of maca root extract on body weight in diabetic obese male rats the results revealed that there was a significant decreased in final body weigh in groups fed maca root extract as compared to positive group. These results agreement with **Zhang *et al.*, (2017**) who noticed HFD-alloxan led to a significant increase in body weight in the experimental mice. Moreover, **Aranaz *et al*., (2019)** . Maca tubers were proposed as dietary supplements for the treatment of chronic diseases characterized by atherogenic lipoprotein profile, liver steatosis,and impaired glucose tolerance, and also for their prevention **(Ve_ce_ra et al. 2007).** It has been reported that maca supplementation prevents lipid and glucose metabolism disorders by regulation of glycolysis/gluconeogenesis-TCA cycle and PPAR-alpha signal activation in rodents fed with a high fat, and high-carb diets **(Wan et al. 2018).** Maca supplementation did not affect weight change. This result is important for the prevention of obesity, as there is no evidence that maca effects weight gain. Our results are compatible with previous data, which was reported Maca to be a considerable polyphenol-rich compound in weight control and provided lower weight gain (Wang et al. 2009)

Table (2): Effect of maca root extract on sex hormones in diabetic obese male rats

Parameters / Groups	Testosterone (ng/ml)	FSH (ng/ml)	LH
Control (-ve)	5.72±0.61a	21.52±0.66a	1.67±0.04a
Control (+ve)	1.22±0.04e	7.60±0.85e	0.92±0.03e
mg Extract of maca 100 root /kg BW	2.25±0.09d	10.38±0.36d	1.17±0.02d
300 mg Extract of maca root /kg BW	3.63±0.13c	15.19±0.39c	1.31±0.01c
500 mg Extract of maca root /kg BW	4.61±0.30b	18.88±0.55b	1.56±0.03b

Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at ($P<0.05$).

Table (2) showed the effect of maca root extract on sex hormones in diabetic obese male rats it can be observed that sex hormones (Testosterone , FSH and LH) were increased significantly in all groups fed on maca extract as compared to positive group .Maca root is an adaptogenic herb that has been shown in studies to improve fertility in both men and women. It contains about 31 different minerals and 60 different phytonutrients. Several studies of rodent models found that maca and its extracts improved the acrosome reaction, sperm motility, and count through the increased structural and functional preservation

of Leydig cells, which produce testosterone (**Valdivia Cuya et al., 2016;Onaolapo et al., 2018; Aoki et al., 2019)**. The potential benefits of maca are related to its androgen-like effects on counteracting CYP-induced changes in the male reproductive system (Onaolapo et al., 2018).

Table (3): Effect of maca root extract on CAT and MDA in diabetic obese male rats

Parameters / Groups	CAT	MDA (nmol/ml)
Control (-ve)	132.17±1.40a	3.10±0.04e
Control (+ve)	72.28±2.06e	6.93±0.21a
mg Extract of maca 100 root /kg BW	84.84±4.75d	4.90±0.08b
300 mg Extract of maca root /kg BW	99.76±1.08c	4.03±0.10c
500 mg Extract of maca root /kg BW	115.66±1.02b	3.60±0.16d

Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at ($P<0.05$).

Table (3) showed the effect of maca root extract on CAT and MDA in diabetic obese male rats the results indicated that CAT increased significantly in groups fed on extracts of maca roots while MDA in groups fed on maca root extract decreased significantly as compared with positive group . Besides the macaenes, macamides are the specific maca ingredients, and the total macamide fraction (TMM) has been shown to have antioxidant and significant antitumor efficacy against the five different cancer cell lines **(Fu et al., 2021).** However, a novel identified polyunsaturated macamide derivative of *Lepidium meyenii* was reported to relieve dextran sulfate sodium-induced colitis in mice as evidence of the beneficial efficacy of Lepidium meyenii in mice intestines **(Zha et al., 2021).** MP can reduce the inflammatory cell infiltration of liver tissue, central venous congestion, and hepatocyte necrosis caused by cyclophosphamide; inhibit weight loss; and improve the activities of liver metabolic enzymes (ALT and AST). MP also improves the activity of antioxidant enzymes and the ability to directly scavenge oxygen free radicals, and may alleviate lipid oxidative damage through antioxidation **(Wenting et al. 2022)**

Table (4): Effect of maca root extract on liver functions in diabetic obese male rats

Parameters / Groups	AST	ALT	ALP
	(µ/L)		
Control (-ve)	56.56±2.64e	26.01±0.41e	77.82±0.77e
Control (+ve)	92.91±1.75a	52.88±1.52a	115.74±2.04a
mg Extract of maca 100 root /kg BW	83.26±1.81b	44.49±2.07b	101.80±1.92b
300 mg Extract of maca root /kg BW	74.88±0.87c	38.56±0.61c	94.33±2.09c
500 mg Extract of maca root /kg BW	63.95±3.57d	30.63±0.86d	86.06±1.96d

Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at (P<0.05).

Table (4) showed effect of maca root extract on liver functions in diabetic obese male rats the results cleared that AST, ALT and ALP decreased significantly in all groups fed on extracts of maca roots as compared to positive group Additionally, maca polysaccharides are promising components with beneficial pharmacological activities on humans, including antioxidant, immunomodulatory, and liver protective activities (Caicai et al., 2018).

Table (5): Effect of maca root extract on lipid profile in diabetic obese male rats

Parameters / Groups	TC	TG	VLDL-C	HDL-C	LDL-C
	(mg/dl)				
Control (-ve)	82.20±2.07e	61.26±3.00e	12.25±0.60e	55.16±2.14a	14.78±3.62e
Control (+ve)	136.15±0.88a	90.68±1.07a	18.13±0.21a	37.33±3.42c	80.68±3.77a
mg Extract of 100 maca root /kg BW	115.56±2.00b	82.07±1.75b	16.41±0.35b	45.10±0.87b	54.05±3.11b
300 mg Extract of maca root /kg BW	107.43±0.97c	74.66±1.17c	14.93±0.23c	49.26±0.89ab	43.23±1.53c
500 mg Extract of maca root /kg BW	95.20±2.04d	68.43±1.09d	13.68±0.21d	52.48±0.88a	29.03±1.49d

Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at (P<0.05).

Data in table (5) shows the effect of maca root extract on lipid profile in diabetic obese male rats the results cleared that TC, TG, VLDL-C and LDL-C decreased significantly in groups fed extract of maca root while HDL-C decreased significantly in groups fed effect of maca root extract as compared with control positive group . our result in agreement with the study as mentioned earlier. In a similar manner to our study, in the HFD diet given mice intestines, the Glut2, Pept1, and membrane receptor FAT-CD6 levels decreased, whereas Glut5 and Fatp4 remain unchanged **(Losacco et al., 2018)**. After the 60 days of study, fat tissue and liver insulin levels decreased in the HFD and HFD + Maca groups in comparison to Control and Maca groups ($p < .001$). Maca group showed a significant IRS1 increase in the fat tissue ($p < .0001$). Leptin levels were the highest in the Maca group and the lowest in the HFD group in the fat tissue ($p < .01$). SIRT1 levels were found the highest in the Maca groups ($p < .01$). These results show the protective and regulatory effectiveness of Maca when fed with a normal or HFD diet. Alkaloids are the major secondary metabolites in maca roots, including macamides, macaridine, β-carboline, imidazole alkaloids, macahydantoins, and macapyrrolins. Macamides, which are the main and unique bioactive alkaloids in maca, have been reported to have various pharmacological activities: neuroprotective, hypolipemic, hypoglycemic, antioxidant, and anti-inflammatory activities (Zhu et al., 2022). These alkaloids in maca roots could represent an appreciated candidate for the discovery and development of new drugs. Furthermore, macaenes which are a class of multi-unsaturated fatty acids in maca roots and represent the second main functional constituent of maca for preventing and improving lipid and glucose metabolism disorders (Wan et al., 2018). In the previous published study, more than 100 compounds were detected based on their accurate mass and fragment ions belonging to several classes: 12 amide alkaloids, 13 imidazole alkaloids, 8 β-carboline alkaloids, 19 macamides, 2 macaridines, 6 macacenes, 1 glucosinolate, 7 organic acids, 26 fatty acids, 2 hydantoin derivatives and 24 miscellaneous compounds **(Ibrahim et al., 2022**). Alkaloids are the major secondary metabolites in maca roots, including macamides, macaridine, β-carboline, imidazole alkaloids, macahydantoins, and macapyrrolins. Macamides, which are the main and unique bioactive alkaloids in maca, have been reported to have various pharmacological activities: neuroprotective, hypolipemic, hypoglycemic, antioxidant, and anti-inflammatory activities **(Zhu et al., 2022)**. Maca roots: A potential therapeutic in the management of metabolic disorders through the modulation of metabolic biochemical markers in rats fed high-fat high-carbohydrate diet (Salma et al 2024)

Table (6): Effect of maca root extract on semen in diabetic obese male rats

Parameters / Groups	Normal (%)	Abnormal (%)
Control (-ve)	87.93±1.13a	12.07±1.13e
Control (+ve)	43.16±0.98e	56.83±0.98a
mg Extract of maca root 100 /kg BW	56.63±1.69d	43.36±1.69b
300 mg Extract of maca root /kg BW	68.93±1.06c	31.06±1.06c
500 mg Extract of maca root /kg BW	77.21±2.25b	22.78±2.25d

Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at ($P<0.05$).

Table (6) showed the effect of maca root extract on semen in diabetic obese male rats the results indicated that semen increased significantly in normal groups fed on extract of maca root while decreased in abnormal groups as compared positive group

Maca treatment was able to increase both the semen volume and sperm motility. However, we failed to find any increase in serum testosterone levels during Maca treatment, which may suggest that either bioavailable testosterone or testosterone receptor binding might be augmented. These results agreed with **(Chacon, 1961**), who claimed that alkaloids from Maca increased estrogen levels. Also, **Manuel Gasco *et al*., (2008)** suggested that estrogen like activity is not present in the extracts of different varieties of Maca. This is confirmed by the failure to increased uterine weight after treatment with Maca. Another possibility is that Maca may act without the participation of androgen mechanism. This seems to be supported by the fact that the weight of seminal vesicle, a target for androgen action, was not influenced by Maca in adult male rats**(Gonzales et al 2001)** Black Maca increased daily sperm production and efficiency, suggesting that Black Maca may become a potential treatment for male infertility **(Gonzalez et al., 2006).** Studies suggest that maca regulates sperm count by maintaining the balance between oxidant and antioxidant status **(Yucra et al., 2008).**

Table (7): Effect of maca root extract on glucose in diabetic obese male rats

Parameters / Groups	Glucose	% of glucose reduction
Control (-ve)	85.49±2.07e	
Control (+ve)	227.26±1.51a	
mg Extract of maca root /kg 100 BW	134.50±2.07b	40.81
300 mg Extract of maca root /kg BW	123.16±2.41c	45.80
500 mg Extract of maca root /kg BW	108.26±1.15d	52.36

Results are expressed as mean ± SE. Values in each column which have different letters are significantly different at ($P<0.05$).

Table (7) shows the Effect of maca root extract on glucose in diabetic obese male rats it can be observed that glucose reduced significantly in all groups fed on extract of maca root as compared to control positive group maca have been demonstrated in several conditions such as prostatic benign hyperplasia, osteoporosis in adults' rats and reduce glycaemia in diabetes mellitus **(Gonzales-Arimborgo et al., 2016)**

Maca supplementation was shown to decrease insulin levels while increasing IRS1, leptin, and antioxidant effective SIRT1 levels in rats fed a high-fat diet **(Gencoglu, 2020)**

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الملخص العربي

تم دراسه تاثير مستخلص جذور الماكا علي تحسن حاله الحيوانات المنويه في ذكور الفئران البدينه المصابه بالبول السكري تم استخدام ٣٥ فار وتقسيمهم الي خمس مجموعات مجموعه ايجابيه سليمه واربع محموعات مصابه بالبدانه والبول السكري مجموعه سالبه وثلاث مجموعات تم تغذيتم علي مستخلص جذور الماكا بمستويات ١٠٠ و٣٠٠ و٥٠٠ مللي ولمده سته أسابيع وتم ذبح الفئران واستخلاص السيرم وعمل التقديرات التاليه صوره الدهون بالدم TCالكوليسترول الكلي TGالجليسريدات الثلاثيه والكلويسترول منخفض الكثافه جدا VLDL–C والكوليسترول مرتع الكثافه HDL–C ووالكوليسترول منخفض الكثافه وظائف الكبد AST و ALT و ALP ووالهرمونات الجنسيه **Testosterone** و**FSH**

وLH ومضدات الاكسده و**CAT** و**MDA** ونسبه الحيوانات المنويه والجلوكوز وأوضحت النتائج تحسن معنوي في وظائف الكبد ودهون الدم الدهون الثلاثيه والكوليسترول الكلي وانخفاض الكلوسترول الضار وارتفاع في الكلوسترول الجيد والهرمونات الجنسيه ونسبه الحيوانات المنويه في الفئران التي تغذت علي مستخلص جذور الماكا بالمقارنه بالمجموعات السالبه

الكلمات المفتاحيه

مرض السكر و نبات الماكا و البدانه و الحيوانات المنويه