Effect of maca(*Lepidium meyenii)* aqueous extract on the epididymal sperms quality and the DNA normality of vasectomized mature mice:model for obstructive azoospermia in men

تاثير مستخلص مكة المائي *(Lepidium meyenii)* على نوعية النطف البربخية وطبيعة الدنا في الفئران مقطوعة الاسهر: موديل تجريبي للرجال المصابين باللا نطفية الانسدادية

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Abstract

This study was aimed to found out the effect of Maca extract on certain sperm function characters and sperm DNA normality in vasectomized and healthy adult mice as a model for men complaining from obstructive azoospermia. Twenty adult male mice were randomly divided into four groups (5males per each group).The first group(GI) was regarded as a negative control that treated with distilled water. Whereas group II was gavage maca extract daily with 1mg/100gm.GropIII,and IV were vasectomized . Mice in GIII were vasectomized without treatment while the mice in group GIV were vasectomized and orally administrated maca dose(1mg/100gm).At the last of experiment(35 days), all the mice were scarified for assessment of certain sperm function parameters . The results revealed that a significant (P<0.05)improvement of grade (A and B) of active sperm motility in mice treated orally with Maca extract was recorded compared with other groups, while grade (C) was significantly increase in vasectomized mice (GIIIgroup) as compared with other healthy mice . DNA fragmentation resulted from fertile mice gavages with maca were reduced compared to healthy non-treated group and other treated and non-treated vasectomized mice (GIII and GIVgroup).Orally administration of animals with Maca extract caused a significant (p<0.05) improvement in the percentage of morphologically normal sperm compared with vasectomized treated and non treated groups.

It is concluded that oral administration of Maca extract caused a significant increment in certain sperm function parameters of vasectomized mice in turn this result can be utilized for obstructive azoospermic men.

Keyword: Maca extract, obstructive azoospermia , certain sperm function parameters, vasectomy

الملخص

هدفت هذه الدراسة الى ايجاد تاثير مستخلص مكة على المعايير الوظيفية للنطف الرئيسية وطبيعة الدنا فيها للفئران مقطوعة الاسهر والفئران السليمة كموديل تجريبي للرجال المصابين بالانطفية الانسدادية . عشرون فار بالغ قسموا بشكل عشوائي الى اربعة مجاميع(خمسة فئران في كل مجموعة).المجموعة الاولى اعتبرت مجموعة سيطرة سالبة وتم اعطائها ماء مقطر فقط .في حين المجموعة الثانية جرعت بمستخلص مكة بشكل يومي 1ملغم/100غم من وزن الجسم .المجموعة الثالثة والرابعة استحدث فيها قطع

الاسهر.الفنران في المجموعة الثالثة لم تعالج سوى بالماء المقطر في حين المجموعة الرابعة جرعت 1ملغم/100غم من وزن الجسم من مستخلص مكة.في نهاية التجربة بعد 35 يوم تم التضحية بالفنران لحساب معايير النطف البربخية الرئيسية.اظهرت النتائج وجود تحسن معنوي (P<0.05)في الحركة التقدمية درجة اي وبي للفنران السليمة المعالجة فمويا بمستخلص مكة بالمقارنة مع المجاميع الاخرى.في حين كان الحركة البطيئة نوع س قد بينت زيادة معنوية في الفنران مقطوعة الاسهر ودون معالجة بالمقارنة مع مجاميع الفنران السليمة الاخرى.كانت نتائج تحطم الدنا في الفنران الخصبة المعالجة بمستخلص مكة اقل بشكل معنوي عن الفنران السليمة غير المعالجة والمجاميع الاخرى مقطوعة الاسهر (المجموعة الثالثة والرابعة). الاعطاء الفموي لمستخلص مكة سبب تحسن معنوي في نسبة الشكلياء الطبيعية عند مقارنة الفنران مقطوعة الاسهر المعالجة وبين غير المعالجة .نستنتج من الدراسة بان التجريع الفموي لمستخلص مكة سبب زيادة معنوية في معايير النطف الرئيسية في الفنران مقطوعة الاسهر وهذه النتيجة يمكن الاستفادة منها في الرجال الذين يعانون من اللانطفية الانسدادية.

الكلمات الدالة: مستخلص مكة، اللانطفية الانسدادية، معايير النطف الرئيسية، قطع الاسهر

Introduction

Maca (*Lepidium meyenii*) is herbaceous part of the crucifer family native to the high Andes of Peru [1]. It is cultured for its fleshy hypocotyl to be used as a root vegetable and a medicinal herb. Maca was cultivated for approximately 1500 years ago [2]. Maca was used by national Andean people as an important diet because of its high nutritional value in addition to its effects on fertility and sexual performance [3] . Maca powder was described to overwhelm many abnormal physiological conditions e.g. anemia and infertility because of its anabolic and aphrodisiac effects, [4,5]. Many ethnobotanical studies found that maca can be used against cancer, sexual and menstrual disorders [6]. Other studies referred to its role in the secretion of hormones, immune stimulation and memory perfection [5].

On the other hand, obstructive azoospermia is one of male infertility problem facing the men worldwide .at the same time, vasectomy operation is a familial technique for determination of birth in men that causes several complications as well. Meanwhile, new surgical methods could lower these complications significantly [7]. It has been noticed that obstructive azoospermia or vasectomy method may change epididymal epithelium function , resulting in poor sperm quality [8]**.** Interestingly , **t**here are few studies search on the effect of maca extract on fertilization capacity of men complaining from obstructive azoospermia. Thus, the present study was applied to study the effect of maca extract on certain sperm function parameters and DNA normality using the mice as a model for obstructive azoospermia in man.

Materials and Methods

The proposal design of this study was submit to Scientific Board and the Scientific Research Ethic Board of the Biotechnology Research Center and the acceptance was obtained in March 2016.

Animals management and study design

The study was enrolled in the Biotechnology Research Center laboratories at AL-Nahrain University through the period from November 2016 till April 2017.Twenty healthy adult male mice, weighed 25-35g were used. Mice were held in plastic cages and placed in a sterile room for two weeks for acclimation.

Room temperature was maintained at 22 - 25 ˚C, with light/dark cycle of 13±2 hrs per day. The shaved wood of the cages was changed twice weekly. The mice were freely had the pellets and water.

- Study protocol

Twenty mature fertile Balb/C male mice were randomly divided into 4 equal groups as the following:

Healthy group (GI): The mice were allowed to received only distilled water.

Healthy treated group (GII: The mice were gavages (1mg/100gm mice body weight) of prepared Maca extract once daily.

Vasectomized group (GIII): The vasectomized mice were gavages distilled water, only.

Treated vasectomized group (GIV):The mice were orally administration (1mg/100gm mice body weight) of maca extract once daily.

All the mice groups were treated for 35 days, which are the days of spermatogenesis in mice.

-Vasectomy

Ten mature males mice were anesthetized by using 0.1ml (10mg/ml)of pentobarbital sodium .Then the scrotum was opened from the intermediate line. The vas deferens was sutured from the two sides using microsurgical set. The area between the sutures was cut by scissors. Then the open area was closed directly from the side of scrotum by simple continuous suture. Antibiotic powder was added locally, leaving the animal individually in the cage for at least 14 days as recommended by Al-Dujaily[9].

Isolation of Epididymal sperm

All the mice involved in this experiments were sacrificed by cervical dislocation .The caudal epididymal region was isolated and placed on the Falcon dish filled with warmed Hams F-12 (one ml) for washing. The isolated caudal epididymal region was cut off many(200) times by microsurgical scissors to have the spermatozoa. Then the suspension was maintained in the incubator(Memmert Company, Germany) for 10 minutes to allow the sperm to swim up and then isolated. The isolated spermatozoa were kept in the incubator for at least 30 minutes. Certain sperm characters namely : Sperm concentration (million/ml), the grades of sperm motility and the percentage of morphologically normal sperm(MNS) were measured as recommended by [10] . Whereas, the percentage of active sperm motility was accounted as recommended by the manual of WHO [11].

Test of DNA normality

The acridine orange fluorescence test was performed for all groups to determine the DNA fragmentation according to the method of Tejada [11].The stock solution of acridine orange was added to 40 ml of (0.1 M citric acid) and 2.5 ml of (0.3 M) $Na_2HPO_4.7H_2O$. Then pH adjusted to (2.5) before staining. Then Carnoy's solution was prepared which is a fixative solution consists of three parts of Methanol, (BDH, England); and one part of Glacial Acetic acid (GCC, U.K.). Carnoy's solution provides a better predictive value for sperm DNA damage by using AO dye [12, 13].

Statistical Analysis:

The data were statistically analyzed using SPSS.21. version for the vasectomized mice treated by Maca extract and non-vasectomized groups. The results of certain sperm function characters were expressed as mean ± standard error and analysis of variance (ANOVA) was used to distinguish the F value .When F value reach the significant level($p<0.05$) ,Least significant test was performed .DNA normality was accounted by using Chi square test [14].

Results

-Sperm concentration($x10^6$/ml):

In Table (1), there was a significant ($P<0.05$) improvement in the mean of sperm concentration(m/ml) of healthy mice group treated with maca (48.11 ±1.32) compared to control non-treated mice group(45.5± 0.23) and vasectomized mice groups(GIII=4.05 ±0.42 and GIV=8.51 ±0.37,respectively). A significant ($P<0.05$) decrease in sperm concentration of vasectomized mice was observed compared to healthy non-treated mice. The mean of sperm concentration in treated vasectomized mice was a significantly ($P<0.05$) increment compared to vasectomized mice without treatment (GIII group) which revealed a significant ($P<0.05$) decrement compared to healthy mice not treated with maca extract.

- Active sperm motility percentage(%):

The grades of active sperms motility (A and B) in vasectomized non-treated mice group(GIII group) was shown a significant ($P<0.05$) reduction compared with healthy and other mice groups .Whereas ,the vasectomized mice gavages with Maca extract was revealed a significant ($P<0.05$) improvement in active sperm motility(Grades A and B) compared with vasectomized non treated mice. A significant($P<0.05$) changes was noticed in active sperm motility of healthy male mice compared with vasectomized mice .There was a significant($P<0.05$) differences in grade A motility of healthy treated group compared with other groups(Table -1).

The progressive movement grade B was significantly ($P<0.05$) decrease in vasectomized mice (GIII group) than that of healthy mice(GI group).Treatment of vasectomized mice with Maca extract resulted in improvement of sperm motility(Grade B).There was a significant ($P<0.05$) increment in grade C motility of vasectomized mice compared to control group (non-treated mice,G1). While the mice treated with Maca extract (i.e.G2 group) showed a significant($P<0.05$) increase in sperm motility Grade C) as compared with healthy mice (GI group) and significant ($P<0.05$) decrease as compared with vasectomized mice treated with Maca extract.

-Morphologically normal sperm (%):

As shown in table (1) the percentage of MNS in vasectomized non-treated mice after 35 days treatment was shown a significant ($P<0.05$) decrement compared to fertile healthy mice .Whereas the measurement of MNS in vasectomized male mice gavages with Maca extract (GIVgroup) shown a significant ($P<0.05$) positive changes compared with vasectomized mice without treatment (GIII group).No significant ($P>0.05$)

difference was found in MNS percentage between healthy mice gavages with maca extract and control group(GI group).

-DNA normality(%):

The result of DNA normality by account DNA fragmentation illustrated in table 2. A significant ($P<0.05$) reduction was noticed in DNA fragmentation in mice treated with maca compared with control and vasectomized non treated mice groups.The result shown that the percentage of DNA fragmentation of vasectomized mice were significantly ($P<0.05$) elevated compared with healthy non vasectomized animals .However, DNA fragmentation in vasectomized mice gavages with maca group showed a significant ($P<0.05$) decrease compared with vasectomized non treated mice group and a significant ($P<0.05$) increase as compared with healthy mice(Table- 2).

Table(1): Effect of Maca extract on certain sperm function parameters in adult mice following 35 days oral administration.

Certain sperm function parameters		Mice groups			
		GI (healthy control)	GII (healthy treated with maca	GIII (vasectomized Mice)	GIV (vasectomized mice treated with maca
Sperm conc. (X 10^6/ml)		45.5± 0.23a	48.11 ±1.32b	4.05 ±0.42c	8.51 ±0.37d
Grades of active Sperm Motility	A	34.16± 0.83 b	69.00± 4.00 a	1.66± 0.69 d	8.50± 0.25 c
	B	25.00± 2.88a	10.00± 0.00b	2.11± 0.29c	8.50± 0.76b
	C	16.66± 1.66c	7.16± 1.16d	80.66 ±1.34a	29.60 ± 0.93b
Morphologically Normal sperm (%)		86.56 ±0.80c	84.67 ±2.03c	55.00 ±2.23a	60.431.20b

-Values are expressed as Means ±SE . (n=5 mice/group).

- different small letters denote significant differences between groups at ($P<0.05$).

Table 2: Effect of Maca on DNA normality (%) of healthy and vasectomized mice

.	Groups			
DNA Status	GI (healthy group)	GII healthy with Maca	GIII vasectomy without Maca	GIV vasectomy with Maca
DNA Fragmentation	6.04±0.56 C	6.23± 0.68 C	20.60± 0.93 A	9.45± 0.97 B

Values are expressed as Means ±SE (n=5 mice/group).

- different capital letters denote significant differences between groups($P<0.05$).

Discussion

The present study noticed an increase in sperm concentration of vasectomized mice treated with maca extract. This finding may resulted from the effect of orally administration of maca extract. It has been recognized that maca powder constituents increase spermatogenesis from primary spermatocyte to secondary spermatocyte through spermatogenesis stages in rats. These observation recommended that maca extract can enhance sperm concentration [15] . Moreover, many studies found a beneficial effect of maca powder to sustain sperm concentration and flagellar movement. Therefore, the powder of maca extract has been believed to enhance certain sperm function characters due to its effect to increase the action on pituitary reproductive hormones i.e FSH and LH hormones [16]. It has been discovered that the maca powder consisted of alkaloids [16,17] which responsible for enhance the fertility effect on both ovaries and testes of the mice and it caused higher sperm concentration in males [18].

The current study believed that sperm count in vasectomized mice was decline may be due to the increase in production of free radicals leading to oxidative stress. It has been found that free radicals has a detrimental effect on testicular function and spermatogenesis process [19]. In addition to that the free radicals affected the ultrastructure of sperm and increase of DNA abnormality.

On the other hand ,the increment in sperm count may be resulted from the gavages of maca extract which may stimulate spermatogenesis process or may be due to its role on the pituitary gland to increase the production of LH hormone and thereby increased sperm concentration [20].It has been reported that the stimulation of FSH and LH action by maca may support the spermatogenesis stages this may induced elevation of sperm concentration [21].

The present study noticed an increase in the grade A and B of active sperm motility of treated mice with maca extract. This observation may be due to the effect of different components found in maca extract which

were provides necessary nutrients like vitamins, amino acid and salts which lead to activation of sperm movement [22], while sperms vasectomized mice showed low percentage of sperm motility grades (A and B) in samples with increased percentage of sperm motility grade C as a result of increased abnormality and dead sperm percentage so that the activity of forward progressive movement decrease.

The attenuation of DNA normality in mice treated with maca and increase of DNA fragmentation in vasectomized mice play important role in the improvement of sperm motility grades (A and B) in healthy treated mice and caused poor motility (grade C) in vasectomized mice due to the role of DNA normality to produce intact sperm with low abnormality [23]. The motility of sperm also affected by oxidative stress and ROS in sperm, thus the components of maca had shown to scavenge free radicals and reduce lipid peroxidation [24].

On the other hand, the motility (grad A and B) of sperm may be decreased in vasectomized mice may be due to decrease of secretory products of epididymus which supply a suitable environment that is considered vital for the attainment of motility and viability of sperm [25].

The decrease in MNS combined with ROS production is an indicator of damage in sperm DNA induced by vasectomy [26,27]. Also, increase of nitric oxide due to free radicals has a abnormal effects with sperm form and DNA denaturation [28]. The result of this research demonstrated a significant reduction in sperm morphology of vasectomized treated mice compared with healthy treated mice. Thus the present study found that maca orally administration will be like antioxidant and can remove the excess free radicals and prevent oxidative stress. It has been recorded that Maca extract led to remove the reactive nitrogen species and sperm morphology return to normal [29]. It has been noticed that sperm production with abnormal function of the epididymal region may occur with time after vasectomy [30].

-DNA normality

Orally administration of maca extract to vasectomized and non-vasectomized mice shown a decrease in the percentage of DNA fragmentation . This observation may be due to productive effect of maca extract on sperm membranes [24]. The maca has been shown to scavenge hydroxyl group as well as superoxide radicals and inhibit their release [31]. Also, the decrease of DNA abnormality in treated groups of mice compared to non-treated mice may be attributed to the quality of CHO in extract which is a great energy source for the sperm active motility with an increase in the percentage of morphological normal sperm and these will effect on number of sperm with normal DNA.

On other hand, vasectomy lead to increase DNA fragmentation in mice. The study finding may be resulted from the effect of free radical on sperm genome that causing high frequencies of single and double-strand DNA breaks [32], thus both superoxide (O^{-2}) and the hydroxyl radical (OH^{-}) are known to mutagenic and cause chromosome deletions, and sister chromatid exchange [33].

It is concluded from present study that Maca extract can be used for improvement of sperm characters in men complaining from obstructive azoospermia.

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