Effect of Royal Jelly on Epididymal Sperms Characters in Vasectomized Mice

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Abstract

Background: Fresh royal Jelly(RJ) is a rich nutrient containing vitamins, proteins, enzymes, amino acids and other minerals. But there are a little information about its role on certain sperm function parameters of obstructive azoospermic men .

Objective: The main aim of the study was to assess the effects of royal jelly on certain sperm functions parameters of obstructive azoospermic men who candidate for one of assisted reproduction technologies (ART)using the vasectomized mice as a model .

Materials and Methods: In this study, 240 mature fertile male mice (8-12) weeks old and 25-35mg body weight were used. The mice were divided into main three groups according to the period of Royal Jelly (RJ) orally administration for one, two and three months. Each group divided into four sub groups :Group one orally administration of free-RJ without vasectomy(R-V-).Group two orally administration of RJ without vasectomy(R+V-).Group three not orally administration of RJ with vasectomy (R-V+). Group four orally administration of RJ with vasectomy (R+V+).In Each group the mice were sacrificed and certain sperm function parameters were recorded .

Results: This study showed a highly significant ($P < 0.05$) improvement in certain sperm function parameters i.e the sperm concentration, active sperm motility grade A and B, with a significant decrease in the proportion of abnormal sperm morphology of vasectomized mice treated with RJ compared to other groups.
Conclusions: It was concluded from the present study that the RJ orally administration enhance strongly certain sperm function parameters of vasectomized male mice. This result indicates the possibility to use this nutrient for male factor infertility causes especially those with obstructive azoospermia.

KEYWORDS: vasectomy, sperm active motility, morphologically abnormal sperms, infertility, Royal jelly.

Introduction

Male infertility is a relatively common problem that affects couples worldwide and obstructive azoospermia is one of them (Barnouti , 1993). It is estimated that approximately 1 out of 6 couples will experience difficulties in reproducing (Zini,*et al.*,1993). Royal Jelly is a product secreted by young nurse worker bees for feeding to the queen, queen larvae and other young larvae. It is derived from the proteins of other nutrients in the pollen ingested by the secreting bees. It is an essential nutrient for young larvae bees and queens and has an important role in

queen's feeding (Hashimoto *et al.*,2005,Karaali,*et al.,*1988).Fresh royal Jelly contains glycolic acid which is mono unsaturated fatty acid that protects skin from dehydration (Markham and Campos,1996). Royal Jelly contains also B- plex vitamins , pantothenic acid (B5), pyridoxine (B6) , acetylcholine and vitamins (A,C,D and B), mineral salts are in descending order: K, Ca, Na, Zn, Fe, Cu, and Mn, , enzymes , hormones , 29 amino acid and antibiotic components . It has abundance of nucleic acid (DNA, RNA). Gelatin which is one of the precursors of collagen, which is a powerful anti-aging element that helps preserve the youth of the body (Hove,*et al*.1985, Malcolm,1995,Kodai,*etal*.2007) . Royal jelly contains considerable amounts of proteins, lipid, sugars and amino acids. Royal jelly also contains vitamin A, B (pantothenic acid, has antioxidant effect), C, D and E, mineral salts are in descending order: K Ca, Na, Zn,Fe, Cu, and Mn, enzymes, antibiotic, components. It also has an abundance of nucleic acid- DNA and RNA. It contains sterols, phosphorous compounds and acetylcholine, which is needed to transmit nerve messages from cell to cell (Hove,*et al.*1985,Kodai ,*et al*.,2007). The RJ is consisted of water (67%), crude protein (12.5%) and simple sugars such as monosaccharides (11%) (Simuth,*et al*.,2003). RJ has always been used as a stimulator of fertility. RJ is effective in improving hormonal equilibrium and fertility in men and women by increasing ova and sperm quality(Lewis,2005) RJ is an important source of para-aminobenzoic acid which increases fertility in women who regularly consume this product for at least 6 months. Together with the pantothenic acid(vitamin B5), this acid induces protein usage for healthy hair growth and for its regimentation and that of the skin's(Suzuki,*et al*.2008). Also Rj contains calcium ,required for calpain enzyme which works to increase calpain activity that it was associated with high sperm motility grade (a+b) which indicate the fertilizable sperms ability(Al-Dujaily, *et al*.,2015, Mossa,*et al*.,2015) .For this purpose, the main aim of the study was to through some light on the effect of royal jelly on certain sperm function parameters of vasectomized mice as a model for obstructive azoospermia.

Materials and Methods

Housing and management of experimental animals: Two hundred Forty mature male(Albino – Swiss) mice of 8-12 weeks age and 25-35 gm weight were obtained from the Animal House at High Institute of Infertility Diagnosis and Assisted Reproductive Technologies /AL-Nahrain University. They were kept in an air conditioned room (25℃) with a photoperiod of 13±2 hours. The animals were housed in box cage of opaque plastic measuring(29×15×12) cm. Its floor covered with wooden shave. Each cage contains four animals with tap water and diet were freely available for the animals. .The animals examined clearly every week , abnormal and sick mice were excluded from the group. Each cage was sterilized with 70% ethyl alcohol once a week regularly. Mature male mice(no=240) were divided into three groups according to the period of orally administration of royal jelly for three independent periods:- one month ,two months and three months . Each period composed of eighty male mice and divided into four subgroups as the following

1-Group No1(T1) : twenty mice as control were treated with normal saline (0.9%)only (R-V-).

2- Group No2(T2) :twenty vasectomized mice were treated with normal saline (R-V+)

3-GroupNo3(T3): twenty vasectomized mice were treated with royal jelly (R+ V+)

4-GroupNo4(T4):twenty mice without vasectomy were treated with royal jelly(R+V-)

These four groups were treated for one month, two months three months ,independently. The dose of RJ was calculated according to animals body weight(100 mg/kg) .

Male vasectomy: Mature fertilized mice were vasectomized according to the procedure mentioned by Al-Dujaily (1996).

Collection and Preparation of Sperms

Mice were sacrificed by cervical dislocation and dissected directly. The caudal of epididymis of each side were isolated and placed in Petri dish containing 1 ml of Hams-F12 medium at 37°C and minced by using microsurgical scissor and forceps.

Microscopic examination:

1-Sperms concentration: Method for estimation of spermatozoa concentration was used by Hinting (1989) and AL- Dujaily (1996) .

2- Sperm motility percentage and grade activity and morphologically Abnormal sperm(MAS): Sperm motility and Abnormal was recorded according to the WHO(1999)in human being and Al-Dujaily *et al*.(2006) in mice.

Results:

Table (1) showed the result of epididymal sperms concentration (million/ml) after one month of nutrition with royal jelly and without royal jelly (control). There was a highly significant ($P<0.05$) increase in sperm concentration (55.20 ±4.20)in group of orally administration of RJ with out vasectomy compared to the result of other groups, .No significant ($P>0.05$) difference was statistically recorded in the mean of sperm concentration between non treated RJ-V+ group and control groups (RJ- V-). In table (1) the results revealed that the sperms concentration of nonvasectomized mice orally administrated with RJ(RJ+V-) for two months showed a significant ($P<0.05$) increase (64.80 ± 4.12) compared to the result of others groups.In table (1) showed the results of epididymal sperms concentration after three months of nutrition with and without royal jelly . There was a highly significant ($P<0.05$)improvement in sperm concentration (65.50 ± 2.26) after treatment with RJ of nonvasectomized mice compared to the result of other groups,There was a significant ($P<0.05$) increase in sperm concentration following two and three month of treatment with RJ in vaseatomized and non vaseatomized mice compared to the treatment for one month.

Table (2) showed the results of epididymal active sperm motility (Grad A) after one month of nutrition with and without RJ .There was a highly significant ($P<0.05$) increase in active sperm motility of RJ treatment without Vasectomy (Grad A) (24.25 ±1.69) compared to the result of other groups.In table (2) the results revealed that the active sperm motility(Grad A)following two months of orally administrated with royal jelly(RJ+V-) showed a significant ($P<0.05$) increase in active sperm motility(Grad A) (27.25± 2.86) compared to the result of others groups. Motility (Grade A) after three month of nutrition with and without royal jelly . There was a highly significant ($P<0.05$)improvement in active sperm motility(Grade A) (29.15 ± 2.05) after treatment with RJ(RJ+V-) compared to the result of other groups. A

significant(P<0.05)improvement in active sperm motility grade A was observe following the period of treatment for two and three months with RJ of vasectomized mice compared to one month .

Table (3) showed the result of epididymal on active sperm motility (Grad B) after one month of nutrition with royal jelly and without royal jelly (control). There was a highly significant (P<0.05) increase in active sperm motility(Grad B) (70.35 ±2.66) compared to the result of other groups. Following two months of orally administrated with RJ, the results shown a significant (P<0.05) increase in active sperm motility(Grad B) in RJ+V- (71.05± 2.47) compared to the result of others groups. After three months of nutrition with and without royal jelly. There was a highly significant (P<0.05)improvement in active sperm motility(Grad B) in R+V- (69.75 ± 3.14) after treatment with RJ(RJ+V-) compared to the result of other groups. As shown in table(3).

Table (4) showed the result of epididymal active sperm motility (Grad A+B) after one month of nutrition with royal jelly and without royal jelly (control). There was a highly significant (P<0.05) elevation in the percentage of active sperm motility(Grad A+ B)non vasectomized mice treatment with RJ(R+ V-) in mice treated with RJ (84.60 ±3.29) compared to the result of other groups. In table (4) the results revealed that the active sperm motility(Grad A+ B)following two months of orally administrated of royal jelly without vasectomy(RJ+V-) showed a significant (P<0.05) increase in active sperm motility(Grad A+ B) (94.30± 2.45) compared to the result of others groups. motility(Grad A+ B)after three month of nutrition with and without royal jelly . There was a highly significant (P<0.05)improvement in active sperm motility(Grad A+ B) in RJ+V- (93.40 ± 2.72) after treatment with RJ(RJ+V-) compared to the result of other groups. The percentage of active sperm motility (A+B) after two and three month of treatment with RJ of Vasectomized and non Vasectomized mice was significantly (P<0.05) higher than that of one month treatment.

In table (5) The results of epididymal morphologically Abnormal sperm(MAS) after one month of nutrition with royal jelly and without royal jelly(control) were shown in table (5) . There was a highly significant (P<0.05) decrease in MAS (1.95 ±0.80) compared to the result of others groups. In table (5) the results revealed that the in Abnormal sperm following two month of orally administrated with royal jelly(RJ)(R+V-) showed a significant (P<0.05) decrease in Abnormal sperm in RJ+V- (2.10 ±0.76) compared to the result of others groups. The percentage of Abnormal sperm after three month of nutrition with and without royal jelly . There was a highly significant (P<0.05) decrease in Abnormal sperm in RJ+V- (2.15 ± 1.31) after treatment with RJ(RJ+V-) compared to the result of other groups. There was a significant (P<0.05) reduce in the percentage of MAS following two and three month of treatment by RJ in vasectomized male mice compared to one month treatment.

Discussion

The results of this study found a positive effect of Royal Jelly and period of nutrition leading to enhance the certain epididymal sperms function parameters (concentration, motility and MAS).The data showed a highly significant increase in sperm concentration this is consistent with what it found in other studies that showed that the treatment with RJ caused a increase in the level of FSH and LH hormones. Follicle

stimulating hormone is required for development of the seminiferous epithelium and initiation and maintenance of the mitotic phases of spermatogenesis, FSH acts on the Sertoli cells by increasing the production of Androgen Binding Protein (ABP) causing proliferation in the spermatogenesis and which in turn is linked with T hormone produced from the Leydig cells, leading to an increase level of T hormone in the testes. Therefore, increases FSH level which causes by RJ leading to promoting the production of ABP from Sertoli cells, and then increasing T hormone level in testes which adversely affect the spermatogenesis (Yin ,et al.,2007). Testosterone is essential for spermatogenesis from spermatogonium to spermatid (West,1997). Royal jelly also contains L-arginine and carnitine amino acid, which essential for spermatogenesis (De Lamirande and Gagnon,1995). It has been reported that RJ increased glutathione with decreased in malondialdehyde levels .In addition to its containing vitamin C, vitamin E and arginine (Bayer,1990). Vitamin E and C are well-documented antioxidant and has been shown to inhibit free-radical induced damage to sensitive cell membranes of the testis and reduced lipid peroxidation in tissue estimation by malondialdehyde. Thus, vitamin E and C significantly decreased MDA, and increased in glutathione level (Ebisch,*et al*.,2006). ..Royal jelly is an anti-hypercholesterolemic and shows anti-inflammatory activity therefore was used commercially for more than 30 years in pharmaceutical products, foods, and cosmetics (Kanber ,*et al.,*2009).The present results inconsistent with the suggestions that amino acids and fatty acids which are present in RJ stimulate directly the secretion of (GnRH) and this stimulates FSH and LH secretions leading to an increase in T hormone secretion (Broukhiq and Martine,1997,Al-Jarah,2002). Royal Jelly contains also hormones , 29 amino acid , antibiotic components,. It has abundance of nucleic acid (DNA, RNA),B- plex vitamins , pantothenic acid (B5), pyridoxine (B6) , acetylcholine and vitamins (A,C,D and B), mineral salts are in descending order: K, Ca, Na, Zn, Fe, Cu, and Mn, and enzymes. Gelatin is one of the precursors of collagen, and have powerful anti-aging element that helps preserve the youth of the body (Hove,*et al*.1985, Malcolm,1995,Kodai,*etal*.2007) .The treatment of adult male rats with RJ concomitantly with hydrogen peroxide caused a significant increase ($P<0.05$) in testicular weight and the body of epididymus, sperm count, testosterone hormone and glutathione level, and decrease in sperm deformity percentage (Hassan,2009)**.**

The data showed a highly significant increase in the percentages of sperm motility and grade activity of forward movement (grade A and grade B) of epididymal sperm sample of mice treated with RJ compare to without RJ. These results were in consistent with the finding of Abd-allah, (2010) report . This may be attributed to the effect of RJ containing motility stimulants such as adenosine and adenosine monophosphate ((AMP) N (1)-oxide), which are already known to enhance the motility of sperm by an inhibiting phosphodiesterase activity, thus enhancing cAMP at the level of the sperm tail and stimulated the phosphorylation of not only mitogen-activated protein kinase (MAPK) but also that of cAMP/calcium - response element-binding protein (Byrd,1981,Vijayaraghavan and Hoskins ,1986,Parrish,*et al*.,1988) . Royal Jelly might enhance motility through partial capacitation of sperm cells as its composition is highly containing calcium ions (Karaali,*et al*.,1988). Consequently , increase of cAMP lead to increase progressive sperm motility. The cAMP plays an important role in the glycolytic path way of the sperm and, through its effect on glycolysis. It can influence the energy generation required for sperm motion (Stannic,*et al*.,2002). Mammalian spermatozoa require exogenous substrates for a

variety of functions, e.g., to preserve intracellular energy reserves, cell components and most notably to support motility (Salisbury,*et al*.,1978). They can obtain energy through mitochondrial oxidative phosphorylation and glycolysis by the consumption of glycolysable sugars, such as glucose, fructose, mannose, and maltose (Salisbury and VanDemark,1961). Fructose is thought to be a major energy source for ejaculated spermatozoa (Nagai,*et al.,*1982) and together with glucose is found in seminal plasma in many mammalian species, but not in dogs; nevertheless, dog sperm can utilize these two sugars. In many species, glucose and fructose have been investigated for the different effects on gametes in terms of metabolizable energy and fertility potential, and the beneficial effects vary between species (Williams and Ford,2001). Glucose and fructose are two of the most commonly used sugars for semen extenders; however, the concentration of these sugars in the extenders for semen varies markedly from 5 mM to about 120 mM (Province,*et al.*,1984,Rote,*et al*.,1999). Additionally, RJ contains calcium which enhances of capacitation process(Abd-Allah,2012). Royal Jelly contains many sugars such as fructose and sucrose (Ross,1999,SabAatini,*et al*.,2009). Fructose is one of the principle energy substrate for spermatozoa (Bearden and Fuquay,1997) and an activator factor of mammal spermatozoa (Rigau,*et al*.,2000). Sucrose are reported to be effective in stabilizing sperm membrane bilayers during storage (Chen,*et al*.,1993)**.** The results of effects of RJ. on sperm morphology will be agreement with AL-Qadhi (1997) who noticed that the effect of most mutagenic agents on sperm morphology (head and tail) are investigated in the five weeks of treatment .This may reflect the sperm exposed to RJ. when it occurs in the spermatogonia which is represent 1st stage of spermatogenesis, this stage more sensitive to be effected by RJ. because of their high mitotic activity which represents a source of all sperm (Tates and Natarajan,1996).There are other reasons make the royal jelly reduces abnormal sperm morphology and increase in sperm concentration , the male reproductive function is under hormonal control ,spermatogenesis is under control of FSH and T hormones, The present results inconsistent with the suggestions that amino acids and fatty acids which are present in RJ stimulate directly the secretion of (GnRH) and this stimulates FSH and LH secretions leading to an increase in T hormone secretion,(Broukhiq and Martine,1997,Al-Jarah,2002,Seely, etal.,1996),while the formation of type A spermatogonia and conversion of primary spermatocyte into secondary spermatocyte (Meiosis I) are dependent on T hormone and the final step of maturation of spermatids are dependent on FSH (Ganong,2005), so the abnormal sperm morphology may reflect on abnormal intratesticular maturation (Acosta,et al.,1998).

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Table (1) : Effect of treatment by Royal Jelly and period of nutrition on sperm concentration of vasectomized and nonvasectomized mice (mean ± SD)

Period of nutrition	Sperm Concentration Million\ml				P value
	RJ-V- Control	RJ+V-	RJ+V+	RJ-V+	
Duration:1month	40.60 ± 1.39 B	55.20 ± 4.20 C	40.60 ± 2.43 B	19.80± 1.46 A	P≤0.05
Duration:2month	40.17 ± 3.30 B	64.80 ± 4.12 C*	44.60 ± 2.26 B*	20.10± 1.47 A	P≤0.05
Duration:3month	40.45 ± 2.06 B	65.50 ± 2.26 C*	45.45 ± 2.24 B*	21.05± 1.20 A	P≤0.05
P value	NS	P≤0.05	P≤0.05	NS	---
* (P≤0.05), NS: Non-significant.					

Values are expressed as mean ±SD ANOVA

- Different capital letters means a significant at P<0.05
- * P<0.05 Significant Differences between duration of treatment
- NS =No significant P >0.05

Table (2): Effect of treatment and period of nutrition on sperm motility Grade A (%) of vasectomized and nonvasectomized mice (mean ± SD)

Period of nutrition	Sperm motility grad A (%)				P value
	Control	RJ+V-	RJ+V+	RJ-V+	
Duration:1month	10.95 ± 1.83 B	24.25 ± 1.69 D	14.20 ± 3.28 C	6.95 ± 0.92 A	(P<0.05)
Duration:2months	10.40 ± 2.01 B	27.25 ± 2.86 D *	16.30 ± 2.07 C	7.05 ± 0.80 A	(P<0.05)
Duration:3months	9.95 ±	29.15 ±	16.76 ±	7.55 ±	(P<0.05)

	1.28 B	2.05 C *	2.36 C	1.02 A	
P value	NS	(P<0.05)	NS	NS	---
* (P≤0.05), NS: Non-significant.					

Values are expressed as mean ±SD ANOVA

- Different capital letters means a significant at P<0.05
- * P<0.05 Significant Differences between duration of treatment
- NS =No significant P >0.05
- AO=Acridine Orange, RJ=Royal Jelly ,V=Vasectomy, +=With, -=Without

Table (3) :Effect of treatment and period of nutrition on motility Grad B (%)of vasectomized and nonvasectomized mice (Mean ± SD)

Period of nutrition	Sperm motility grad B (%)				P value
	RJ-V- Control	RJ+V-	RJ+V+	RJ-V+	
Duration:1month	62.25 ± 1.44 D	70.35 ± 2.66 C	34.25 ± 2.38 B	31.55± 2.13 A	P≤0.05
Duration:2month	61.35 ± 2.43 D	71.05 ± 2.47 C	37.95 ± 2.29 B	32.95± 1.90 A	P≤0.05
Duration:3month	60.70 ± 1.58 D	69.75 ± 3.14 C	35.75 ± 2.89 B	31.10± 2.25 A	P≤0.05
P value	NS	NS	NS	NS	---
* (P≤0.05), NS: Non-significant.					

Values are expressed as mean ±SD ANOVA

- Different capital letters means a significant at P<0.05
- * P<0.05 Significant Differences between duration of treatment
- NS =No significant P >0.05

Table(4) Effect of treatment and period of nutrition in Grad A+B (%)of vasectomized and nonvasectomized mice(mean ± SD)

Period of nutrition	Motility Grade A+B (%)				P value
	RJ-V- Control	RJ+V-	RJ+V+	RJ-V+	
Duration:1month	73.25 ± 2.44 D	84.60 ± 3.29 C	59.00 ± 4.17 B	38.45± 2.42 A	P≤0.05
Duration:2month	72.50 ± 4.30 D	94.30 ± 2.45 C*	64.75 ± 3.12 B*	40.05± 2.08 A	P≤0.05
Duration:3month	70.15 ± 2.95 D	93.40 ± 2.72 C*	63.05 ± 2.90 B*	38.65± 2.66 A	P≤0.05
P value	NS	P≤0.05	P≤0.05	NS	---
* (P≤0.05), NS: Non-significant.					

Values are expressed as mean ±SD ANOVA

- Different capital letters means a significant at P<0.05
- * P<0.05 Significant Differences between duration of treatment

Table(5) Effect of Royal Jelly and period of nutrition on morphologically Abnormal sperm % of vasectomized and nonvasectomized mice (mean ± SD)

Period of nutrition	Morphologically Abnormal sperm (%)				p value
	Control	RJ+V-	RJ+V+	RJ-V+	
Duration:1month	4.95 ± 0.80	1.95 ± 0.80	39.15 ± 2.43	63.50 ± 2.20	1.122 *
Duration:2month	5.10 ± 1.30	2.10 ± 0.76	30.75 ± 1.66	64.55 ± 2.55	1.101 *
Duration:3month	5.05 ± 0.86	2.15 ± 1.31	28.50 ± 2.01	63.80 ± 1.83	1.016 *
P value	NS	NS	*	NS	---
* (P≤0.05), NS: Non-significant.					

Values are expressed as mean ±SD ANOVA

- Different capital letters means a significant at P<0.05
- * P<0.05 Significant Differences between duration of treatment
- NS =No significant P >0.05