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EFFECT OF ROYAL JELLY ON DNA INTEGRITY OF EPIDIDYMAL SPERMS IN VASECTOMIZED AND NON- VASECTOMIZED MICE

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ABSTRACT

Background: Fresh royal Jelly contains vitamins, proteins, enzymes, amino acids and other minerals. But the role of royal jelly on sperm DNA normality was unknown. **Objective:** The main aim of the study was to assess the effects of royal jelly on certain sperm DNA integrity of obstructive azoospermia for using in assisted reproduction technologies(ART). **Materials and Methods:** In this study, 240 mature fertile male mice (8-12) weeks old were used, and divided in two main three groups according to the period of Royal Jelly (RJ) orally administration .Each group divided into four sub groups one ,two ,three month :namely :Group one without orally administration of RJ and 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 period the male 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, morphologically abnormal sperms (MAS)and DNA abnormal using acridine orange test, Especially in the case of those with normal sperm parameters with a significant decrease in the proportion of abnormal sperm morphology with a decrease clearly and significantly (P <0.05) the results of epididymal sperm showed a highly

significant (P<0.05) decrease in DNA abnormality by using Royal jelly Following one, two, three month of RJ orally administration of Vasectomized male mice. The best result that improve sperm characteristic was following RJ administration for two months. **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 to increase the chance of pregnancy through one of assisted reproductive technologies programs.

KEYWORDS: Royal jelly, obstructive azoopermia, vasectomy, acridine orange, DNA abnormal sperms.

INTRODUCTION

Fertility is defined as the capacity to reproduce or the state of being fertile[1] while Infertility is a relatively common problem that affects couples worldwide. It is estimated that approximately 1 in 6 couples will experience difficulties in reproducing.[2] Royal Jelly is a cream product secreted by young nurse worker bees for feeding to the queen, queen larvae and other young larvae. It is totally synthesized by the bees in the hypopharangeal and mandibular glands and 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. It has also been shown that royal jelly has different types of biological activity in various cells and tissues of animal models.[3,4]

Fresh royal Jelly contains glycolic acid which is mono unsaturated fatty acid that protects skin from dehydration.[5] 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 is one of the precursors of collagen, which is a powerful anti-aging element that helps preserve the youth of the body.[6, 7 , 8] Royal jelly contains considerable amounts of proteins, lipid, sugars and amino acids. The RJ is consisted of water (67%), crude protein (12.5%) and simple sugars such as monosaccharides (11%).[9] RJ has always been used as a stimulator of fertility. Moreover, RJ is effective in perimenopausal symptoms, osteoporosis, improving hormonal equilibrium and fertility in men and women by increasing ova and sperm quality.[10,11] Also RJ contains calcium that required for calpain enzyme activity that associated with high sperm motility

grade (a+b) which indicate the fertilizable sperms ability.[12,13] For this purpose, the main aim of the study is to through some light on the effect of royal jelly on DNA integrity of vasectomized mice as a model of obstructive azoospermia in men .

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.

Grouping of mice: Mature male mice(no=240) were divided into three main groups according to the period of orally administration of RJ 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-Group No3(T3): twenty vasectomized mice were treated with royal jelly (R+ V+)
4-Group No4(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) .Mature male mice were sacrificed and their reproductive organs were isolated after 1,2 and 3 months of treatment. Sperms were collected from caudal epididymis and allocated into culture medium. The sperm suspension was incubated for 1 hour at 37C under 5% CO_2.

Male vasectomy: Mature fertilized mice were vasectomized according to the procedure mentioned by Al-Dujaily.[15]

Preparation of acridine orange Solution

As described by Tegada[19] the stock solution was prepared by.

1-Na2Hpo4 .7H2o = 0.588g in 10 ml distal water.

2-Citric acid = 2g in 100 ml D.W.

3-Acridine Orange powder = 1g in 1000 ml D.W.

Ten ml of AO was added to 40 ml citric acid and 2.5 ml of Na2HPo4 .7H2O .In Darkroom. at 4 c stored. pH 2.5.

Acridine orange protocol

Smears were prepared from each sample on the slides and allowed to air-dry for about 20 minutes. Then, the slides were fixed in Carnoy's solution for at least 3 hours to overnight at 4ºC. Carnoy's solution was prepared daily. After that, the slides, were removed from the fixer and allowed to air-dry for a few minutes before staining. After being placed on a slide holder, a 2-3 ml of the stain were spread over each slide for 5 minutes. They were gently rinsed in a stream of distilled water. The slides were allowed to dry, mounted and a 22x50 mm glass cover slip was put upon it. Slides were read on the same day of staining with a (40X) objective lens on a fluorescence microscope (BEL Italy) image , which was equipped with an excitation filter of 460-490 nm and barrier filter of 520 nm. The nuclei of at 300 spermatozoa from each sample were examined and scored as fluorescing green, red or yellow. Spermatozoa displaying green fluorescence were recorded as normal, whereas sperm heads displaying yellow-red fluorescence were considered as abnormal.[19]

RESULTS

Results of DNA integrity by acridine orange test

Table (1): showed the result of DNA integrity of epididymal sperm preparation after one month of nutrition with and without RJ . There was a highly significant ($P<0.05$) increase in sperm DNA integrity (92.75 ±1.73) in RJ+V- group compared to the result of other groups(without royal jelly(control) (87.20 ± 1.78) and Group without Royal Jelly and vasectomy (RJ-V+) (87.90± 1.58) .While no significant ($P>0.05$) difference was noticed in vasectomized mice treated with RJ(RJ+V+)(92.90 ± 1.81).

In table (1) the results revealed that the percentage of sperm DNA normality following two months of orally administrated of royal jelly(RJ)(RJ+V-) showed a significant ($P<0.05$) increment in sperm DNA normality of RJ+V- mice (97.55 ±1.28) compared to the result of control group (89.40 ±2.24) , group with RJ+V+(92.55 ± 1.65) and group RJ-V+ (88.25 ±1.72).

The percentage of sperm DNA integrity after three months of nutrition with and without RJ was shown a highly significant (P<0.05) improvement in RJ+V- mice (95.20 ± 2.20) than that of other groups: without RJ(control) (89.40 ± 2.47),group RJ+V+ (92.80± 1.66) and Group without Royal Jelly and Vasectomy (R-V+)(84.85 ± 2.77).

The treatment with RJ for one ,two and three month did not show any significant (p> 0.05) differences between vasectomized and non vasectomized mice.

Table (1): Effect of Royal Jelly and period of nutrition on sperm DNA integrity(%)of vasectomized and nonvasectomized mice (Mean ± SD)

Period of nutrition	DNA integrity (%)				p value
	RJ-V- (Control)	RJ+V-	RJ+V+	RJ-V+	
Duration:1month	87.20 ± 1.78 B	92.75 ± 1.73 C	92.90 ± 1.81 D	87.90± 1.58 A	P<0.05
Duration:2month	89.40 ± 2.24 B	97.55 ± 1.28 C	92.55 ± 1.65 D	88.25± 1.72 A	P<0.05
Duration:3month	89.40 ± 2.47 B	95.20 ± 2.20 C	92.80 ± 1.66 D	84.85± 2.77 A	P<0.05
P value	3.572 NS	6.803 NS	2.559 NS	5.074 NS	---
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

Correlation coefficient between parameters study

Table (2) showed the correlation between different certain sperm function parameters and DNA normality in the current study. There was no correlation between DNA normality using Acridine orange test and other parameters namely; 1-active sperm motility,(grad B) in three month r=0.02 , r= 0.08 ,r=0.05 respectively. 2-Morphologically abnormal sperm through the three months r= - 0.15 , r= - 0.07 ,r= -0.16 respectively.

There was a highly significant (P<0.01) positive correlation between DNA normality and sperm concentration through the three months of nutrition with RJ groups r= 0.36 , r = 0.25 ,r =0.33 respectively.

There was high significant (P<0.01) positive correlation between DNA normality and active sperm motility,(grad A) following one month and two months (r = 29 , r =29 respectively) while the treatment for three months was shown no significant(p>0.05) correlation(r = 0.21). There was a high significant (P<0.01) positive correlation between DNA normality and grade (A + B) after one month (r = 0.29). While there was a significant correlation after three months (r = 0.25). No significant correlation was observed between DNA normality and grade(A +B) following two months of treatment(r = 0.14).

Table (2) Correlation coefficient between parameters study/per month

Parameters correlated	Correlation coefficient (r)		
	Month 1	Month 2	Month 3
Acrdine Orange & Conc.	0.36 **	0.25 *	0.33 **
Acrdine Orange & Grad A%	0.29 **	0.29 **	0.21 *
Acrdine Orange & Grad B%	0.02 NS	0.08 NS	0.05 NS
Acrdine Orange & Grad A+B%	0.29 **	0.14 NS	0.25 *
Acrdine Orange & Abnormal sperm	-0.15 NS	-0.07 NS	-0.16 NS
* (P≤0.05), ** (P≤0.01), NS: Non-significant.			

Effect of Royal Jelly and period of nutrition on sperm DNA integrity

The Royal Jelly gave best result regarding certain sperm function parameters of epididymal sperms when decrease the percentage of DNA abnormality .This result may be caused by synergism effect of Royal Jelly. The decrease in DNA abnormality following RJ can be explained by the lipid content of the sperm cell membrane, which contains a high proportion of polyunsaturated fatty acids. This fatty acid will effect positively to decrease the production of lipid peroxidation which is the source of Reactive oxygen *species* (*ROS*).[20,21] At the same time Royal Jelly contains different antioxidant with different cryoprotectant such as glucose, sucrose, fructose, and maltose. All will revealed a positive effect on DNA normality.[9] The other explanation is that RJ may affect positively on the DNA integrity through the period of nutrition leading to decrease the percentage of abnormal sperms with absent in leukocytes and any cell may generate ROS. It has been reported that two potential sources of ROS can be generated: spermatozoa [22 ,23] and leukocytes.[24, 25] It was suggested that RJ might maintain the acrosomal integrity as its composition is highly variable containing hormones and different vitamins which decreases oxygen radicals and increase the number of

scavengers needed in the seminal plasma to counteract the effects of ROS on lipid peroxidation and prevent spermatozoal damage.[26]

Finally, according to the properties of RJ, DNA integrity was significantly improved in vasectomized and non vasectomized sperms which may emphasize that RJ can be used as either nutrient supplement or treatment of choice for obstructive azoospermic men to lowered the negative effects of long storage of epididymal sperms before any procedure such as fine needle aspiration to accomplished one of assisted reproductive techniques(ART).

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