Effect of Phoenix dactylifera Pollen on In Vitro Sperm Activation of Infertile Men

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ABSTRACT

The objective of this study is to improve sperm motility in vitro of infertile men by using Phoenix dactylifera Pollen extract (P. dactylifera pollen) as motility stimulant substances that can improve assisted reproductive technologies (such as artificial insemination) in future. Semen was collected from 25 infertile men who involved in this current study. Each semen sample was divided into two portions. One part was considered as a control and in vitro activated by using culture medium only. The other portion was considered as treated portion and in vitro activated by adding P. dactylifera pollen extract (0.5mg) to the culture media. Certain sperm function parameters were examined before and following in vitro activation using simple layer technique. The results revealed highly significant increment (P<0.01) in the percentage of total sperm motility grade (A+B+C) and progressive sperm motility grade (A) with a significant improvement (P<0.05) in the percentage of progressive sperm motility grade (B) when using P. dactylifera pollen medium in comparison with control medium after 10 and 30 minutes incubation. It is concluded from the results of the present study that adding the 20% P. dactylifera pollen extract to the culture medium of the in vitro sperm activation leads to an improvement in the sperm motility.

INTRODUCTION

Pollen grains carry the male genetic material, by a variety of means, for gamogenesis in the plant kingdom. Pollen applications in the rites, and its uses in traditional and herbal medicine, have been recorded throughout history. They have been used in the treatment of sexual incapacity and weaknesses in the Arab World[1]. Suspension of P.
Phoenix dactylifera pollen is a herbal mixture that is widely used as a folk remedy for curing male infertility in traditional medicine [2]. The male flowers of date palm are also eaten directly by people as a fresh vegetable to enhance fertility [3]. The effect of *P. dactylifera* pollen on sperm parameters and reproductive system of adult male rats was studied and the results indicated that the consumption of *P. dactylifera* pollen suspensions improved the sperm count, motility, morphology and DNA quality with a concomitant increase in the weights of testis and epididymis [4]. Other report is indicating that *P. dactylifera* contain estradiol and flavonoid components that have positive effects on the sperm quality [5]. It has long been believed that date increase the sexual ability in man [3]. Phytochemical studies of *P. dactylifera* pollen grains revealed the presence of steroidal saponin glycoside [6]. In another study the results showed that date extract caused a significant increase in sperm cell concentration (total count) and motility, on other hand *P. dactylifera* pollen extract decreases sperm cell count but increases the percentage of motile sperm [7]. Furthermore, administration of the pollen grains extract caused a decrease in epididymal sperm with tail abnormalities that would interfere with sperm motility and the highest dose retained normal epididymal sperm number. These findings suggested the preventive role of pollen grains against the chemotherapeutic-induced infertility in males [8].

**MATERIALS AND METHODS**

Semen samples from 25 infertile men were obtained during their attendance to IVF Institute of Embryo Research and Infertility treatment, University of Al-Nahrain. The mean ages of patients was 39 ± 0.96 years with a range of 25 to 59 years. The sample of seminal fluid were collected after 3 to 5 days of abstinence directly into a clean, dry and sterile disposable Petri-dishes by masturbation in a room near the laboratory [9]. After liquefaction time, analysis of semen samples before and after swim up preparation in vitro were done to determine the sperm count and sperm motility percentage. At least 200 spermatozoa in five different microscopic fields were evaluated from each sample. The specimens was examined in details by macroscopic and microscopic examinations using standardization of WHO [9].

**Preparation of *P. dactylifera* pollen extract**

The method for preparation of *P. dactylifera* pollen extract was aqueous method, in which, 1000gm of *P. dactylifera* pollen in granular powder moistened with boiling water and percolated until the *P. dactylifera* pollen was exhausted, filtrated and evaporated until black pillar mass having a characteristic of sweet taste powder was prepared.
The *P. dactylifera* pollen extract was stored in well closed container protected from light and moisture described by Al–Ahliya Flavors and Fragrances Co. Ltd. (IRAQ).

**Preparation of *P. dactylifera* pollen for *in vitro* sperm activation**

The concentration of *P. dactylifera* pollen working solution was prepared by adding 0.5 mg of *P. dactylifera* pollen extract to (10ml) of phosphate buffer solution in plastic test tube contained broad spectrum antibiotic (Ampicillin 0.004 gm) to prevent bacterial growth. The media used for activation contained 20% of *P. dactylifera* pollen working solution by adding 2ml of *P. dactylifera* pollen working solution to (8ml) of FertiCult flushing media. The solution was filtered using Millipore 0.45 µM and have been fixed at pH 7.4-7.8 at 25°C.

**Experimental design**

Each semen sample was divided into two portions. One part was considered as a control and *in vitro* activated by using culture medium only. The other portion was considered as treated portion and *in vitro* activated by adding *P. dactylifera* pollen (0.5mg) to the culture media. Certain sperm function parameters were examined before and following *in vitro* activation using simple layer technique. Then incubated at 37°C for 10 and 30 minute. A drop 10µl taken from the top layer was aspirated by pipette to be examined under 40X-objective. The effect of prepared media on sperm parameter were studied.

**Statistical Analysis:** The data were expressed as mean ± SE. Analysis of variance (ANOVA) test and least significant test (LST) were used to detect the significance between the variables.

**RESULTS AND DISCUSSION**

The present study has investigated the positive effect of adding *P. dactylifera* pollen extract to culture medium by *in vitro* direct activation technique on certain sperm function parameters following 10 and 30 minutes (table.1). However, The mean of sperm concentration in before activation portion has been highly significant (P<0.001) elevation compared to both control and treated portions. While the total sperm motility (A+B+C) and progressive sperm motility grade A and B were highly significant (P<0.001) decrease in before activation portions compared with both control and treated portions after 10, 30 minutes. On other hand, a significant (P<0.05) increase have been demonstrated in the percentages of sperm motility grade C in before activation compared with samples activated with *P. dactylifera* pollen extract, while no significant (P>0.05) difference was observed in control semen.
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sample when compared with results before activation. This finding may have resulted from the activation technique when only the sperm with active motility have swim-up to the upper layer of the medium [10]. In addition to the contains of culture medium that used for activation select only intact sperm to swim up to the upper surface of the medium.

The data have revealed that the addition of P. dactylifera pollen extract to sperm activation medium caused significant (P<0.05) improvement in the mean of sperm concentration, total sperm motility (A+B+C) and progressive sperm motility grade A and B compared with control portions through the incubation for 10, 30 minutes. Furthermore, the addition of P. dactylifera pollen extract to sperm activation medium caused a significant (P<0.05) reduction in the percentages of sperm motility grade C in treated portion compared with the results of control portions. This is probably due to the fact that P. dactylifera pollen contain concentrations of phytochemicals and nutrients and are rich in carotenoids, flavonoids and phytosterols [11]. Moreover, they are good source of protein, amino acids, vitamins, dietary fiber, fatty acids, enzymes, hormones and minerals [12].

In vitro activation with P. dactylifera pollen extract increase the percentage of sperm motility. The constituent of P. dactylifera pollen (i.e. phytoestrogens) may sustain this observation. It is well known that the sperm express estrogen receptors, adding P. dactylifera pollen leading to increase the influx of Ca^{2+} and in turn increase cAMP, which has shown to be a very important factor in sperm motility percent and grade activity [13]. In addition P. dactylifera pollen mainly contains cholesterol, rutin, carotenoids, as estrone which is known to exhibit gonadotrophin activity in the rat [14]. Furthermore, P. dactylifera pollen contain proteins, vitamins (E, A, folic acid and others) mineral such as potassium, magnesium, calcium, manganese and iron [12], all of these substances stimulate sperm motility and the grade activity of forward movement. In vivo observation found that administration of the pollens extract for male mice caused a decrease in epididymal sperm with tail abnormalities that would interfere with sperm motility, and the highest dose retained normal epididymal sperm number [8]. These findings suggest the preventive role of the pollen grains against the chemotherapeutic-induced infertility in males. In another study [7] the results show that P. dactylifera pollen extract decreases sperm cell count but increases the percentage of motile sperm in male guinea pig. Oral administration of P. dactylifera pollen suspension at doses of 120 and 240 mg/kg body weight improved the sperm count, morphology and DNA quality with a concomitant increase in the weights of testis and epididymis [4]. In another study, it has been looked into the antioxidant and hypolipidemic effects of pollen extracts.
(cernitins) on male rabbits and Wister rats. They demonstrated the reduction of malondialdehyde (MDA), total cholesterol, and triglyceride content under the influence of cernitins, indicating their antioxidant properties [15]. Also, Al-Shagrawi [16] showed that the existence of some flavonoids, such as quercetin, rutin and β-amirin in the pollen grains, plays a role in preventing the peroxidation of fatty acid in the body. In several studies a close relationship between pollen antioxidant bioactivity and phenolic compounds has been reported [17,18,19]. Carotenoids such as beta-carotene and lycopene also form an important component of the antioxidant defense [20]. Beta-carotenes protect the plasma membrane against lipid peroxidation [21]. Nass-Arden et al., [22] concluded that lipid peroxidation activity has a major role in loss of sperm motility during time of incubation. Other results reported that rutin and quercetine have been recognized to act against apoptosis (programmed cell death) [23]. Therefore, *P. dactylifera* pollen may be protect the sperm against apoptosis leading to increase the percentage of normal sperm form.

*In vitro* studies showed that vitamins E and C are major chain–breaking antioxidants in sperm membranes and appears to have a dose dependent protective effect [24] and this may be the role of *P. dactylifera* pollen when added through activation program *in vitro*. It is concluded from the results of the present study that adding the 20% *P. dactylifera* pollen to the culture medium of the *in vitro* sperm activation leads to an improvement in the sperm motility *in vitro*. 
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Table-1: The effect of P. dactylifera pollen on sperm motility of infertile men following in vitro activation.

<table>
<thead>
<tr>
<th>Certain sperm Characters</th>
<th>Before activation</th>
<th>Incubation time</th>
<th>In vitro activation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>After activation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>controls</td>
<td>treated</td>
</tr>
<tr>
<td>Sperm concentration (10^6 sperm/ml)</td>
<td>*</td>
<td>10 min</td>
<td>7.2 ± 1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min</td>
<td>12.7 ± 2.3</td>
</tr>
<tr>
<td>Total sperm motility (%) (A+B+C)</td>
<td>*</td>
<td>10 min</td>
<td>77.0 ± 3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min</td>
<td>79.0 ± 2.4</td>
</tr>
<tr>
<td>Sperm motility grade (A) %</td>
<td>9.2 ± 0.89</td>
<td>10 min</td>
<td>33.1 ± 6.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min</td>
<td>36.0 ± 4.9</td>
</tr>
<tr>
<td>Sperm motility grade (B) %</td>
<td>20.1 ± 2.6</td>
<td>10 min</td>
<td>26.1 ± 2.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min</td>
<td>29.5 ± 5.5</td>
</tr>
<tr>
<td>Sperm motility grade (C) %</td>
<td>20.0 ± 2.5</td>
<td>10 min</td>
<td>18.2 ± 3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 min</td>
<td>19.4 ± 1.7</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
No. of Samples per portion = 20.
* P<0.001 Highly significance between before and after activation of control and treated portions.
** P<0.05 Significance between before and after activation of control and treated portions.
*** P<0.05 Significance between before and after activation of treated portions.
+ P<0.001 Highly significance between treated portion and control portion.
++ P<0.05 Significantly different between treated portion and control portion.

REFERENCE

7. Omar M.M.; Shanawany A.A.; Iamail A. & Mohsen M.K. The effect of palm pollen grains and date extracts on the


