

Effect of Different Levels of Plants (*Thymus vulgaris* and *Ziziphus spina_christi*) on Semen Diluents for Awassi Ram.

تأثير المستويات المختلفة لنبات الزعتر *Thymus vulgaris* والسدر *Ziziphus spina_christi* على مخفف المنى للكباش العواس Awassi ram

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Abstract:

Male infertility presents a particularly vexing clinical problem. Clinically infertility can be defined as the inability of a couple trying to conceive to do so within one year. Medicinal plants are increasingly recognized worldwide as an alternative source of efficacious and inexpensive medications to synthetic chemo-therapeutic compound. Various medicinal plant extracts have been tested for their activity both in male and female animal models. This work was performed to evaluate the effects of hot water extracts of two plants (*Thymus vulgaris* and *Ziziphus spina_christi* on Awassi ram sperm activity. Three different concentrations of the two plants were used (0.001, 0.05, and 0.5 mg), and the activity of the sperm were evaluated at the time: 0, 24, 48, 72 hours of experiment. Results showed a significant decrease in sperm activity associated with the treatment by the concentrations 0.005 and 0.01 mg of *Ziziphus*, and 0.01 mg of *Thymus vulgaris*. Effect of time also showed a significant decrease in sperm activity with the progression of time. The 0.001 of *Thymus vulgaris* is the only concentration that showed a significant increase in sperm activity.

الخلاصة:

يعتبر عدم الخصوبة في الذكور من المشاكل الطبية المزعجة. يعرف عدم الخصوبة طبيا على انه عدم قدرة الزوج على احداث الحمل خلال عام كامل. ازداد استخدام النباتات الطبية في العالم كمصدر بديل فعال وغير مكلف للعلاج بدلا عن المركبات الكيميائية المصنعة. اختبر العديد من المستخلصات النباتية المنوعة في مجال عدم الخصوبة للذكور والاناث لحيوانات التجربة. انجز هذا البحث لتقييم كفاءة مستخلصات الماء الحار لنباتي الزعتر *Thymus vulgaris* والسدر *Ziziphus spina_christi* على فعالية النطف في الاكباش العواس. تم استخدام ثلاث تراكيز مختلفة من النباتين (0.001, 0.05, and 0.5 mg), بعدها تم تقييم فعالية النطف في الاوقات (0, 24, 48, 72) ساعة من التجربة. اظهرت النتائج انخفاض معنوي في فعالية النطف في المجاميع المعاملة بالتراكيز (0.005, 0.01) من نبات السدر, والتركيز (0.01 mg) لنبات الزعتر. كما بينت النتائج وجود انخفاض معنوي لتركيز النطف مع تقدم وقت التجربة. ان التركيز 0.001 mg لنبات الزعتر هو التركيز الوحيد الذي اظهر زيادة في تركيز النطف.

Introduction:

Male infertility presents a particularly vexing clinical problem. It is customary to define infertility clinically as the inability of a couple trying to conceive to do so within one year. Male infertility is commonly due to deficiencies in semen and the semen quality is used as surrogate measure of male fecundity [1], [2].

Medicinal plants are increasingly used worldwide as an alternative source of efficacious and inexpensive medications to synthetic chemo-therapeutic compound, and high proportion of the world's population rely on plants for their primary Health care [3,4]. Various medicinal plant extracts have been tested for their antifertility activity both in male and female animal models. Profiles of 50 plants species with antifertility, reported in the literatures from 1994-2010. The profiles presented include information about the scientific name, family, the degree of antifertility activity and the active agents [5].

Thyme (*Thymus vulgaris* L., family: Lamiaceae) is one of plant species which are known to have positive antimicrobial, antioxidative and anti-inflammatory effects [6,7,8]. It was found that thyme leaves water extract and powder achieved the highest increase in antioxidant enzymes superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GPx), and significant reduction in malondialdehyde (MDA) in aged rats brains [9]. Also thyme was found to have significant enhancement of male fertility parameters [10]. *Ziziphus spina_christi* (family: Rhamnaceae) also play an important role in many biological processes, it's well known to have positive hypoglycemic, insecticidal, antimicrobial, antioxidative and anti-inflammatory effects [11, 12, 13, 14].

Materials and Methods : **(Hot water Extract Preparation)**

The Powder of (*Thymus vulgaris* and *Ziziphus spina_christi*) were mixed with hot water after cooling water to temperature less 50 °c. The mixing was done by using a vibrator for 15 minutes. Then the mixture was left for 24 hours. The filtration process was done by using four layers of gauze. Then the leaky was put in a dishes which is heated to 40 °c inside an oven for drying the extract. The dry raw solid material for extract then waited and the required concentrations were prepared.

Semen was collected from one of the rams Awassi 3- year- old and weight 70 kg then diluted the semen by Tris- egg yolk diluent (1:10) .then distributed randomly into three groups first one control, second group contain (*Thymus vulgaris*) extract and the third group contain (*Ziziphus spina_christi*) extract on three concentration (0.001, 0.005, and 0.1 mg) each.

Samples were refrigerated gradually to 5 Celsius and placed in a glass flask containing water in 37 Celsius then kept in the refrigerator.

The activity of the sperm were evaluated at the time: 0, 24, 48, 72 hours of experiment. Depending on individual motility of the sperm (0-100) [15].

Statistical analysis was conducted by using general leaner model for SAS system and the comparative between means was conducted by using Duncan's Multiple Range Test [16].

Results:

The effects of the different concentrations of both plants were shown in the table-1, which revealed a significant decrease in sperm activity associated with the treatment by the concentrations 0.005 and 0.01 mg of *Ziziphus spina_christi*, and 0.01 mg of *Thymus vulgaris*. The effect of the time of treatment by both plants on sperm activity also showed a significant decrease in sperm activity with the progression of time as in the table-2. Table-3 represents the comparison between the effects of the two plants on sperm activity according to the concentration and time. It showed that, 0.001 *Thymus vulgaris*, caused a significant increase in sperm activity along the period of treatment compared with the control and the other concentrations of the same plant and the other plant of this study.

Table-1 : The effects of different concentrations of *Thymus vulgaris*. and *Ziziphus spina_christi* on sperm activity.

Plant	Concentration	Sperm activity
<i>Thymus vulgaris</i>	0.0	43.75 A
<i>Ziziphus spina_christi</i>	0.0	45.00 A
<i>Thymus vulgaris</i>	0.001	58.75 A
<i>Ziziphus spina_christi</i>	0.001	40.00 A
<i>Thymus vulgaris</i>	0.005	39.00 A
<i>Ziziphus spina_christi</i>	0.005	12.00 B
<i>Thymus vulgaris</i>	0.01	12.50 B
<i>Ziziphus spina_christi</i>	0.01	2.00 B

Table-2 : The effects of the time of treatment by *Thymus vulgaris*. and *Ziziphus spina_christi* on sperm activity.

Plant	Time	Sperm activity
<i>Thymus vulgaris</i>	0.0	43.75 A
<i>Ziziphus spina_christi</i>	0.0	45.00 A
<i>Thymus vulgaris</i>	24	62.50 AB
<i>Ziziphus spina_christi</i>	24	36.50 C
<i>Thymus vulgaris</i>	48	7.75 D
<i>Ziziphus spina_christi</i>	48	15.25 D
<i>Thymus vulgaris</i>	72	7.50 D
<i>Ziziphus spina_christi</i>	72	3.50 D

Table-3 : Comparison between the effects of *Thymus vulgaris* and *Ziziphus spina_christi* on sperm activity.

Plant	Time / Concentration	0.0	24	48	72
<i>Thymus vulgaris</i>	0.0	90 B	85 C	0 K	0 K
	0.001	90 B	85 C	30 G	30 G
	0.005	85 C	70 D	1 K	0 K
	0.01	40 F	10 I	0 K	0 K
<i>Ziziphus spina_christi</i>	0.0	95 A	85 C	0 K	0 K
	0.001	50 E	50 E	50 E	10 I
	0.005	25 H	10 I	10 I	3 JK
	0.01	5 J	1 K	1 K	1 K

Discussion:

Results showed a significant decrease in sperm activity associated with the treatment by *Thymus vulgaris*. and *Ziziphus spina_christi* extracts, except the 0.001 mg of *Thymus vulgaris* which caused a significant increase in sperm activity. These results may reflect the undesired effects of both plant and these are not the only plants that showed similar effects on male fertility [5], or the effects may be due to the sensitivity of concentration as the 0.001 mg of *Thymus vulgaris* caused a significant increase, and this considered good sign for the study of different other concentrations. The significant increase of sperm activity caused by 0.001 mg of *Thymus vulgaris* may put this plant in the list of other plants that found to enhance the male fertility [17], [18].

The effects of both plants may be related to the direct effects on spermatogenesis (weight of testes, body of epididymis, percentage of live/dead sperms and epididymal sperm count), or may be related to immunoinfertility and the formation of antisperm antibodies, which can be a major factor of male fertility [10], [19].

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