Effect of Aqueous and Ethanolic Extracts of Tribulus terrestris, Phoenix dactylifera and Nasturtium officinale Mixture on Some Reproductive Parameters in Male Mice

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Abstract:

The present investigation was conducted to evaluate the effect of the crude extracts mixture of three plants (*Tribulus terrestris*, *Phoenix dactylifera* and *Nasturtium officinale*) on semen quality,sex hormones and reproductive performance of mature male mice. A group of 25 male mice given 150mg/kg/day of the powder of the plants mixture with the food for four weeks and another three groups of 25 animals each given intraperitoneal injection from each of the aqueous and ethanolic extracts with a doses 75, 150, and 300mg/kg/day for two weeks. A remarkable increase in sperm concentration and motility with a decreased abnormal morphology was obtained in the experimental groups. A significant increase in hormones level were recognized in most groups. The results of mating untreated females with treated males of the four experimental groups revealed a decreased gestation period and an increased litter size. The results showed a dose dependent pattern of activity and the effect of the extracts were enhanced with increasing the dose level. The ethanolic extract being the more effective extract in all parameters.

Keywords: Tribulus terrestris, Phoenix dactylifera, Nasturtium officinale, Reproduction, mice.

Introduction:

In the past, medicinal plants were considered as the only form of health care readily available to the majority of human population [1]. At present, it is estimated that about 80% of the world population relies on botanical preparations as medicine to meet their health needs [2]. In recent years, in view of their beneficial effects, the use of spices and herbs has been gradually increasing in developing countries[2].*Tribulus terrestris* L. (TT) is a member of the plant family Zygophillaceae. The fruit is regarded as tonic, diuretic and aphrodisiac. It is also used to treat urinary disorder, impotency and heart disease. The seeds are recommended in hemorrhages, kidney stone and gout [3]. The extract of TT contains protodioscin (PTN), a steroidal saponin that has been extensively used for the treatment of various ailments, such as urinary [4] and cardiovascular[5] disorder. Administration of TT to human and animals improves libido and spermatogenesis [6, 7]. TT has a proerctile effect [8]. *Phoenix dactylifera* (Pd) is a member of the plant family Arecaceae. Date extracts have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentration of testosterone, FSH and LH in rats. The pollen grains of date palm have been used by Egyptians to improve fertility. Date

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pits have been included on the animal food to enhance growth [9]. *Nasturtium officinale* (No) is a member of the plant family Brassicaceae.The complete German Commission E Monographs improved the No for the catarrh of the respiratory tract. In Germany, it is also used to treat urinary tract infections in children [10]. The powdered leaves are used in India as an expectorant to treat bronchitis and a number of conditions affecting human liver [11].The fresh herb is used as a blood purifier [12]. The present investigation was conducted to evaluate the possible effect of the crude extracts mixture of the three plants on semen quality, sex hormones and reproductive performance of mature male mice.

Materials and Methods*:*

Animals:

Mature Swiss albino male mice, nine weeks old and 25-30 gm in weight, were used in this study. The animals were kept under standard conditions of temperature (25c^{o}) and lighting period (14hrs light/10hrs dark). Food and water were supplied ad libitum.

Plant materials:

Tribulus terrestris and Pd which were collected from Baghdad (Al-Khadimiya area) and identified by the Iraqi National Herbarium Staff and No seeds which were obtained from herbalist, were mixed in a proportion of TT (aerial parts, 40%), Pd (pollen grain, 30%) and No (seeds, 30%) and powdered by using an electrical grinder. Preparation of the aqueous and ethanolic crude extracts of the plants materials was conducted according to Mattar, 2005[13].

Treatmen:

1-Oral administration:

Two groups of 25 animals were used. G_1 considered as a control group and fed the normal food only; G_2 was the experimental group which was given the crude mixture of plants added with the normal food in a dose of 150mg/kg/day for a period of four weeks. Fifteen animals from each group were killed at the end of the fourth week for semen analysis (sperm concentration, motility and morphology). Blood serum was used to evaluate hormone levels (testosterone, FSH, LH) by the end.

of the fourth week by using a gamma counter and a special hormonal kits[14].

2-Intraperitoneal(ip) injection of the aqueous and ethanolic extracts:

Four groups (per each extract) of 25 animals were treated for a period of two weeks. G_1 was the control group given either water or olive oil injection (0.5ml) daily, G_2, G_3 and G_4 received an ip injection in a dose of 75, 150 and 300mg/kg/day respectively from each extract. Fifteen male mice from each group of both treatment were killed at the end of the 1st and 2nd week of treatment for semen analysis (sperm concentration, motility and morphology), and evaluation of hormones level "LH, FSH and testosterone". Ten male mice from each group of both treatments (oral and ip) were mated, at the end of treatments, with untreated mature females at the ratio of one male /three females to check the reproductive performance. Timing of pregnancy was performed by observing the presence of vaginal plug which is regarded to be day one of pregnancy.

Collection of blood sample:

Blood samples were collected under light anesthesia by heart puncture using a 22-19 mm gauge needle. Serum for hormonal assay was obtained by centrifugation for 10 minutes at 3000 rpm and kept at (-20C^{o}) until use.

Microscopic examination of epididymal sperms:

Examination of epididymal sperms and calculation of sperm concentration, sperm motility and sperm morphology was conducted according to Al-Dujaily 1996[15]

***Statisticalanalysis*:**

Computerized statistical analysis was performed using the SPSS (statistical package of Social Sciences) version 10 under windows XP-2000(Inc, Chicago, IL,USA) computer soft ware and the use of excel program. Values reported are means ±SE .Experimental results were statistically analyzed using the t-test, with P values less than 0.05 considered significant, less than 0.01 considered highly significant [16].

Results:

The effect of Oral administration of plants mixture powder:

The effect of dry powder of plants mixture on epididymal sperm parameters is shown in table 1. A significant ($P<0.05$) increase in sperm concentration was found after the 2nd and 4th week of treatment in comparison with the controls. A significant ($P<0.05$) increase in sperm motility was shown after the 2nd week of administration and a highly significant ($P<0.01$) increase was obtained after the 4th week of treatment compared to controls. No significant difference in abnormal sperm morphology was found after the 2nd week of treatment, whereas a highly significant ($P<0.01$) decrease appeared after the 4th week in comparison with the controls.The effect of feeding plants mixture powder on hormonal levels is shown in table 2. A highly significant ($P<0.01$) increase in LH and FSH levels and a significant ($P<0.05$) increase in testosterone level were found after the 4th week of treatment as compared with the control group.Table 3 shows the effect of feeding plants mixture on reproductive performance. A highly significant ($P<0.01$) increase in the number of fetuses and a significant ($P<0.05$) decrease in the gestation period was found in the treated group in comparison with the controls.

The effect of ip injection of the aqueous extract:

The effect of ip injection of the aqueous extract on sperm parameters is shown in table 4. No significant difference in sperm concentration after the 1st week of treatment in G_2 compared to G_1, whereas, G_3 and G_4 showed a highly significant ($P<0.01$) increase in comparison with G_1 after the 1st week of treatment. A significant ($P<0.05$) increase obtained in G_2 and a highly significant ($P<0.01$) increase resulted in G_3 and G_4 after the 2nd week of treatment compared to G_1 with respect to the same parameter. After the 1st week of treatment, G_2 showed a significant ($P<0.05$) increase in the % motility and a highly significant ($P<0.01$) increase in G_3 and G_4 compared to G_1. After the 2nd week, there was a significant ($P<0.05$) increase in G_2, G_3 and G_4 in comparison with the control group G_1. G2 showed a significantly ($P<0.05$) decreased abnormal sperm morphology, whereas G_3 and G_4 showed a highly significant ($P<0.01$) decrease after the 1st week of treatment compared to G_1. After the 2nd week, a highly significant ($P<0.01$) decrease was observed in G_3 and G_4 compared to G_1.

The effect of ip injection on hormonal levels is shown in table 5. G_2 showed no significant difference in LH, FSH and testosterone levels after the 1st and 2nd weeks of treatment except a significant ($P<0.05$) increase in testosterone level after the 2nd week of treatment compared with G_1. G_3 revealed a significant ($P<0.05$) increase in LH level after the 1st and 2nd week and a significant ($P<0.05$) increase in testosterone level after the

2nd week in comparison with G_1. G_4 showed a highly significant (P<0.01) increase in LH level and a significant (P<0.05) increase in FSH and testosterone levels after the 1st week of treatment in comparison with G_1, whereas after the 2nd week, a significant (P<0.05) increase in FSH level and a highly significant (P<0.01) increase was observed with respect to LH and testosterone levels in comparison with G_1. Table (6) shows the effect of ip injection of the aqueous extract on fertility capacity. After the 2nd week of treatment, G_2 and G_3 showed a significant (P<0.05) increase in number of fetuses and a significant (P<0.05) decrease in gestation period with a highly significant (P<0.01) increase in No. of fetuses and a highly significant (P<0.01) decrease in gestation period in G_4 in comparison with G_1.

Table 1: **Effect of feeding crude mixture of TT, Pd and No on some semen parameters in mice.**

Group / Week	G_1			G_2		
	Conc./ml	Motile%	Abnormal morphology	Conc./ml	Motile%	Abnormal morphology
2nd	40.6± 2.28x10^6	70.6± 0.89	66.4± 2.2	a 50.8± 1.95x10^6	a 75.2±1.78	62.8±1.28
4th	44.8± 2.44x10^6	78.6± 1.19	62.8± 1.1	a 60.8± 3.10x10^6	b 94.6±0.54	b 23.6±1.61

Values = mean ±SE

[a]P <0.05 compared to the control group.

[b]P <0.01 compared to the control group.

Table 2: Effect of feeding crude mixture of TT, Pd and No on hormonal level in mice.

Group / week	G_1			G_2		
	LH mIU/ml	FSH mIU/ml	Testosterone pg/ml	LH mIU/ml	FSH mIU/ml	Testosterone pg/ml
4th	1.34±0.03	1.57±0.01	3.6±0.12	b 2.17±0.01	b 1.98±0.01	a 4.3±0.14

Values = mean ±SE.

[a]P <0.05 compared to the control group.

[b]P <0.01 compared to the control group.

Table 3: Effect of feeding crude mixture of TT, Pd and No on reproductive performance in mice

Group	Average No. of fetuses	Gestation period (day)
G_1	5.06±0.59	20.8±0.56
G_2	b 9.0±0.92	a 19.8±0.67

Values = mean ±SE.
[a]P <0.05 compared to the control group.
[b]P <0.01 compared to the control group.

Table 4: Effect of ip injection of aqueous extract of TT, Pd and No on some semen parameters in mice.

Week / Group	After 1 week			After 2 weeks		
	Concen./ml	Motile%	Abnormal morphology	Concen./ml	Motile%	Abnormal morphology
G_1	44.6± $3.09x10^6$	76.6±1.30	63.2±1.95	44.0± $3.29x10^6$	82.0±1.74	50.2±3.76
G_2	52.8± $1.03x10^6$	a 84.2±1.38	a 53.4± 1.30	a 58.8± $2.9x10^6$	a 87.0± 1.74	48.2± 1.95
G_3	b 62.2± $1.27x10^6$	b 90.4±1.70	b 43.8±1.17	b 69.0± $1.87x10^6$	a 94.0±2.24	b 37.6±1.51
G_4	b 71.0± $2.18x10^6$	b 92.8±1.50	b 31.0±3.48	b 77.8± $1.1x10^6$	a 97.2±1.10	b 22.8±2.14

Values = mean ±SE.
[a]P <0.05 compared to the control group.
[b]P <0.01 compared to the control group

Table 5: Effect of ip injection of aqueous extract of TT, Pd and No on hormonal level in mice

Week / Group	After 1 week			After 2 week		
	LH mIU/ml	FSH mIU/ml	Testosterone Pg/ml	LH mIU/ml	FSH mIU/ml	Testosterone Pg/ml
G_1	1.34±0.02	1.58±0.01	3.54± 0.09	1.35±0.01	1.58±0.01	3.64±0.09
G_2	1.40±0.01	1.61±0.02	3.76±0.17	1.42±0.01	1.64±0.01	a 4.42±0.08
G_3	a 2.25±0.03	1.86±0.02	4.26±0.11	a 2.38±0.08	1.93±0.02	a 4.84±0.05
G_4	b 2.80±0.01	a 1.95±0.01	a 4.68±0.13	b 3.34±0.05	a 1.98±0.01	b 5.42±0.08

Values = mean±SE.
[a]P <0.05 compared to the control group.
[b]P <0.01 compared to the control group.

Table 6: Effect of ip injection of the aqueous extract of TT, Pd and No on reproductive performance in mice

Group	Average No. of fetus	Gestation period (day)
G_1	5.4±0.34	21.8±1.01
G_2	a 6.3±0.50	a 20.4±0.43
G_3	a 6.4±0.44	a 20.4±0.33
G_4	b 7.7±0.40	b 20.2±0.22

Values = mean ±SE.
[a]P <0.05 compared to the control group.
[b]P <0.01 compared to the control group.

The effect of ip injection of ethanolic extract:

The effect of ip injection of ethanolic extract on epididymal sperm parameters is shown in table (7). Treatment of the animals with the dose levels used (G_2 G_3 and G_4) showed a highly significant (P<0.01) increase in sperm concentration after the 1st and 2nd weeks of treatment in comparison with the control group G_1. G_2 showed a significant <0.05) increase in the % motility after the 1st and 2nd weeks of treatment as compared to G_1 ,whereas G_3 and G_4 showed a highly significant (P<0.01) increase after the 1st and 2nd weeks of treatment compared to G_1.There was a significant (P<0.05) decrease in the abnormal sperm morphology in G_2 after the 1st week of treatment compared to G_1, while there was a highly significant (P<0.01) decrease appeared in G_3 and G_4 after the 1st and 2nd weeks of treatment compared to the control group.The effect of ip injection of ethanolic extract on hormonal levels is shown in table (8). G_2 showed no significant differences with respect to all hormones after the 1st and 2nd weeks in comparison with control group. In G_3, A highly significant (P<0.01) increase in LH level and a significant (P<0.05) increase in FSH and testosterone levels were found after the 1st week of treatment. The same trend was found in the 2nd week, which showed a significant (P<0.05) increase in LH and FSH levels and a highly significant (P<0.01) increase in testosterone level in comparison with G_1. G_4 showed a highly significant (P<0.01) increase in LH level and a significant (P<0.05) increase in FSH level after the two periods in comparison with the control group, as well as a significant (P<0.05) increase was found after the 1st week and a highly significant (P<0.01) increase after the 2nd week in testosterone level as compared with the control group. After two weeks of treatment, G_2 showed a significant (P<0.05) increase in number of fetuses and a significant (P<0.05) decrease in gestation period, whereas, G_3 and G_4 exhibited a highly significant (P<0.01) increase in the number of fetuses and a significant (P<0.05) decrease in gestation period in comparison with the control group G_1 (table 9).

Table 7: Effect of ip injection of ethanolic extract of TT, Pd and No on semen parameters in mice.

Week / Group	After 1 week			After 2 weeks		
	Concen./ml	Motile%	Abnormal morphology	Concen./ml	Motile%	Abnormal morphology
G_1	44.6± 3.09x10^6	76.6±1.30	63.2±1.95	44.0± 3.29x10^6	82.0±1.74	50.2±4.76
G_2	b 58.8± 1.13x10^6	a 85.2± 1.18	a 51.4± 1.10	b 61.8± 1.8x10^6	a 88.0± 2.33	a 43.2± 1.25
G_3	b 60.2± 1.05x10^6	b 90.0±0.89	b 40.6±1.13	b 72.1± 1.18x10^6	b 94.5±2.04	b 33.6±1.37
G_4	b 77.0± 1.58x10^6	b 94.8±1.57	b 28.6±1.06	b 80.8± 1.51x10^6	b 97.5±2.10	b 21.8±1.26

Values = mean ±SE.
[a]P <0.05 compared to the control group.
[b]P <0.01 compared to the control group.

Table 8: Effect of ip injection of ethanolic extract of TT, Pd and No on hormonal levels in mice.

Week / Group	After one week			After two weeks		
	LH mIU/ml	FSH mIU/ml	Testosterone Pg/ml	LH mIU/ml	FSH mIU/ml	Testosterone Pg/ml
G_1	1.38±0.01	1.58±0.01	3.48±0.21	1.73±0.01	1.57±0.02	3.54±0.07
G_2	1.42±0.02	1.63±0.01	3.76±0.05	1 1.46±0.01	1.66±0.02	4.06±0.11
G_3	b 2.29±0.01	a 1.88±0.02	a 3.98±0.04	2.37±0.02	a 1.96±0.01	b 4.54±0.04
G_4	b 2.92±0.03	a 1.98±0.01	a 4.22±0.07	b 3.50±0.04	a 2.16±0.05	b 5.58±0.13

Values = mean ±SE.
[a]P <0.05 compared to the control group.
[b]P < 0.01 compared to the control group

Table 9: Effect of ip injection of the ethanolic extract of TT, Pd and No on reproductive performance in mice.

Group	Average No. of fetus	Gestation period (day)
G_1	5.4±0.44	21.8±0.61
G_2	a 6.26±0.40	a 21.26±0.39
G_3	b 7.46±0.34	a 20.46±0.34
G_4	b 8.53±0.44	a 20.22±0.39

Values = mean ±SE.
[a]P <0.05 compared to the control group.
[b]P <0.01 compared to the control group.

Discussion:

The three different methods of treatment used in this study although differ to a certain degree in their effects on the different parameters, but they showed that the plants extracts have a good activity on reproduction in male mice. The significant increase in sperm concentration, motility and improvement in sperm abnormal morphology agrees with the report traced in the literature that dealt with

this aspect on laboratory animals and human [8, 9, 17, 18]. These changes can be explained on the basis that the plants mixture contains many materials which act as a potent antioxidant like vitamin C, E, A and B which may protect sperm membrane against lipid peroxidation thus lowering the percentage of dead sperm and maintaining normal sperm morphology. The mixture also contains many trace elements (Ca, Mg, Mn, Na, K, Zn ions and others), these minerals especially Ca^{++} is known to inhibit the enzyme phosphate diesterase , which prevents cAMP degradation and consequently increasing sperm motility and sperm hyperactivity [19]. The presence of zinc in the mixture leads to improvement of the sperm count, motility and morphology, because it involved in hormone metabolism, RNA and DNA organization, protein synthesis, cell division biomembranes and nuclear chromatin stabilization [20]. The significant changes in serum levels of the reproductive hormones studied, FSH, LH and testosterone played an important role in increasing sperm concentration. Boukhliq and Martin, 1997 [21] have shown that amino acids and fatty acids may stimulate directly the secretion of Gn-RH which in turn stimulates FSH and LH secretion. FSH is known to be the major hormone responsible for sperm production and maturation [22]; it is also an important factor in the final stages of epididymal sperm maturation [23]. FSH level showed a significant increase at different groups of treated animals. This hormone regulates the Sertoli cell function including increasing the production of androgen binding protein and the latter works in keeping a high concentration of testosterone in the testes which is necessary for normal spermatogenesis and sperm maturation. FSH is also an important factor in the initiation and continuation of spermatogenesis [24]. The improvement in sperm motility may have been brought about by increasing intercellular cAMP, which is known to be a very important factor in stimulating sperm motility [25]. The PTN present in the mixture leads to a direct increase in LH and dehydroepiandrosteron (DHEA) levels which leads to increase in testosterone level , and thus the stimulation of spermatogenesis with an increase in concentration of spermatozoa [8,26]. TT may increase fertility by a direct action on germinal and Sertoli cells by improving spermatogenesis, it may improve libido by increasing the level of LH which activates the production of testosterone from the Leydig cells by increasing the androgen receptor sensitivity or by stimulating the enzyme 5-alpha-reductase which increases the conversion of testosterone into dihydrotestosterone (DHT). In addition to its activity on sexual performance.
The potassium ion present in the mixture may have an important role in increasing the LH and testosterone levels by affecting the pulsatil release of Gn-RH from the hypothalamus which is an important factor in regulating androgenic status and fertility [27, 28]. The presence of vitamin A is required for maximal production of testosterone and vitamin B helps to optimize macronutrient metabolism, maximize muscle mass and decrease the serum levels of homocysteine, cholesterol and C-reactive proteins, marker of heart disease and inflammation in the body. Decreasing inflammation helps to decrease the cortisol level and thus increase the anabolic effect of the mixture [29]. The glycosides present in No acts in the same way because it has an anti-inflammatory effect [30]. All these factors lead to improvement in

the concentration and motility of sperm and in some cases increase in the volume of ejaculation [29] and this may explain the significant increase in the result of reproductive performance in this study which is due to the increase in sperm number, motility, and the improvement in morphology and erection function. The significant decrease in gestation period is the most common complication of multiple pregnancies which leads to preterm labor and premature rupture of the membrane. This hypothesis originated from the observation of the length of gestation versus litter size which demonstrated a direct correlation between uterine stretching and the initiation of parturition [31]. In conclusion the results showed a dose dependent pattern and the effect of the extracts were enhanced with increasing the dose. The ethanolic extract being the more effective extract in all parameters. The possible reason for the variation in the effect between aqueous and ethanolic extracts could be due to the difference in the active principles present in the two extracts.

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تأثير المستخلص المائي ومستخلص الايثانول لمزيج نباتات الكطب وطلع النخيل وبذور الرشاد على بعض المعايير التكاثرية في ذكور الفئران

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الخلاصة:
اجريت الدراسة الحالية لتقييم الفعالية البايولوجية لمزيج مستخلص خام لثلاث نباتات هي الكطب وطلع النخيل والرشاد على نوعية السائل المنوي والهورمونات الجنسيه وخصوبة ذكور الفئران. عوملت مجموعة من الحيوانات وعددها 25 بجرعة 150 ملغم/كغم/يوم بمسحوق مزيج النباتات بخلطه مع الطعام لفترة اربعة اسابيع فيما استخدمت مجموعة اخرى وعددها 25 كمجموعة سيطرة اعطيت الطعام الاعتيادي فقط، وتم حقن ثلاثة مجاميع اخرى (لكل مستخلص) داخل البريتون بكل من محلول المستخلص المائي ومستخلص الايثانول وبجرع 75، 150و300 ملغم/كغم/يوم لفترة اسبوعين واستخدمت مجموعتي سيطرة بنفس الاعداد عوملت احداهما ب 0.5 مليليتر زيت الزيتون والاخرى ب 0.5 مليليتر ماء اعتيادي. لوحظت زيادة في تركيز وحركة النطف مع انخفاض في الشكلياء الغير الطبيعية لتلك النطف، كما لوحظت زيادة معنوية في مستويات جميع الهورمونات وفي معظم المجاميع المعاملة مقارنة مع مجاميع السيطرة. اظهرت نتائج مزاوجة الاناث غير المعاملة مع الذكور المعاملة وفي جميع المجاميع انخفاض في فترة الحمل وزيادة في عدد الولادات. اظهرت النتائج بأن نمط التأثير يعتمد على الجرعه مع ارتفاع تأثير المستخلصات بزيادة الجرعة وكان مستخلص الايثانول الاكثر تأثيرا في معظم المعايير التي تم اختبارها.