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Effects of Qat, (*Catha edulis*) extracts in combination with dimethoate on sperms quality of male mice

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ABSTRACT

The present study was aimed to evaluate the effects of Qat, *Catha edulis*, extracts in combination with dimethoate at the dosage level of 14 mg/kg on mice reproduction. Qat extracts alone or combined with dimethoate were orally given by gavage to male mice every other day (total of 30 treatments). Seminal fluid analysis (percentage of sperm viability, percentage of sperm motility, sperm grade motility and sperm concentration) was recorded. The percentage of sperm viability, was not significantly affected by the treatment with either 2.5 or 5.0 g Qat extract / Kg body weight compared to the control. The percentage of sperm viability, percentage of sperm motility, sperm grade motility and sperm concentration were significantly ($p < 0.05$) affected after treatment with 14 mg dimethoate /kg compared with the control. The percentage of sperm viability, percentage of sperm motility, sperm grade motility and sperm concentration were significantly ($P<0.05$) affected after treatment with both tested doses of water Qat extracts plus 14 mg dimethoate/kg) compared with the control. The results clearly indicate that dimethoate/Qat extracts mixture had more dramatic reduction in seminal fluid than dimethoate alone, or water Qat extracts, alone in descending order. This high risk demonstrated in Qat plants sprayed with the insecticide (e.g. dimethoate) necessitates the public awareness especially between youngsters to avoid the fatal habit of Qat chewing and also to avoid the adverse effect of chewing Qat leaves on the productivity and fertility. It is also the responsibility of the health officers, educators, the media and growers to revise their philosophy and to implement a new policy in which

Qat is no longer encouraged as an acceptable social habit since it affects our health.

Key words: Qat, *Catha edulis,* extracts, dimethoate, combination, laboratory testing, seminal fluid analysis, sperms, male mice

INTRODUCTION

Qat (*Catha edulis*, Forskal) (Celastraceae) is an evergreen tree which grows at high altitudes extending from East to Southern Africa, as well as Afghanistan, Yemen and Madagascar (Krikorian 1984). The earliest scientific report concerning Qat was in the eighteenth century by the botanist Peter Forskal, (Baasher 1980). The alkaloid fraction is the active constituent of Qat and cathine (d-norpseudoephedrine). The search for a more powerful stimulant led to the isolation of a new alkaloid (a-aminopropiophenone) from the fresh leaf (Halbach 1972). Other constituents include tannins, amino-acids and a significant amount of ascorbic acid, magnesium and beta-carotene (Kalix, 1984; Kalix & Braeden, 1985; Kennedy, 1987). Cathinone, cathine and norephedrine are related to ephedrine, a stimulant present in prescription and over the counter medications and products, e.g. herbal dietary supplements for weight loss and treatment of asthma (Greenway 2001), and together they are known as phenylpropylamines (PPAs), with cathine, norephedrine and ephedrine being PPAs. Previous studies on PPAs have focused on developing methods to accurately detect these compounds in blood and urine samples (notably cathinone and its metabolites: (Brenneisen *et al.*, 1986; Toennes and Kauert, 2002; Toennes *et al.* 2003). or in the CNS, trying to understand how PPAs exert their psychoactive effects. Data from studies in the CNS suggested that these compounds can act at noradrenaline (norepinephrine) transporters; for example, ephedrine has been shown to act as an adrenergic agonist, activating adrenergic receptors both directly and indirectly, via carrier-mediated exchange with norepinephrine (Rothman *et al.*, 2003). An early study investigating the role of cathinone in brown tissue thermogenesis obtained evidence suggesting that beta adrenergic receptors might be involved in the responses obtained Tariq *et al.*1989).

Relatively few studies have addressed possible effects on reproductivity and these have focused on the male since the recreational use of Qat is more prevalent in males than females (Al-Motarreb *et al.*, 2002). Looking for possible mutagenicity caused by cathinone (Qureshi *et al.*, 1988), orally administered aqueous solutions of the compound to adult male mice for 6 weeks, then tested their fertility by mating with females for 2 weeks. At the highest dose tested (40 mg/kg body weight) males appeared to be sterile in the first week of testing, but effects were short-lived, with none detected in the second week, and the underlying biology was very unclear. In another study, administration by injection of pure cathinone was said to cause degenerative changes in testicular morphology and reductions in plasma levels of testosterone in male rats (Islam *et al.*, 1990), but this route of administration is quite different from that occurring when Qat leaves are chewed. In a study involving humans (El-Shoura *et al.*, 1995), semen parameters in two groups of Yemeni males, Qat 'addicts' and 'non-addicts', were compared; sperm concentration, morphology and motility were reported to be significantly poorer in the 'addicts'. However, the age ranges in both groups were wide, there were no details on amounts of Qat ingested by the addicts and there was no information on the men's intrinsic fertility, making it difficult to draw sound conclusions.

Due to the high economic importance of Qat as a cash crop, its producers are always keen to use pesticides heavily for protection from various pests to ensure healthy Qat foliage for fresh consumption. The misuses use of pesticides, especially on Qat is one of the great dangers threatening man and environment in Yemen. Several studies have shown evidence that pesticides has strong relations with cancer, human mutations, congenital malformations, inhibition of body immunity and endocrinal disturbances (Al-Hadrani and Thabet, 1999). The majority used a single pesticide in each application, but in other cases mixtures of two or more of the pesticides were used in combinations (Thabet and El-Sebae, 2008).

The risk assessment of human exposure to Qat alone, or Qat contaminated with pesticide residues reveals the hazards of both acute and long-term exposure to the qatamines in Qat alone or combined with the hazardous insecticides such as the plant systemic dimethoate insecticide (Thabet, 1994).

The present study was conducted to evaluate the toxic effect of Qat water extracts and/ or its combinations with $1/10^{th}$ and $1/5^{th}$ LD_{50} of the dimethoate insecticide on seminal fluid analysis.

MATERIALS AND METHODS

Pesticide used: Dimethoate

- Chemical name: *O,O*-dimethyl S-[2-(methylamino)-2- oxoethyl]. phosphorodithioate
- Common name: dimethoate
- Source: Dimethoate (40% E.C.) introduced by Cheminova Co. BAEF-Group
- Acute oral toxicity: LD_{50} for mice 140 mg/Kg (according to Eto, 1974).

The tested concentrations in the present study depended on that LD_{50}

Preparation of Qat extracts: The fresh samples of stem tips and leaves of Qat, *Catha edulis* (Forskal.) were obtained from Yemen. Five hundred grams of Qat stem tips and leaves were homogenized in 100 ml of distilled water (D.W.) using a Polytron homogenizer for one hr. Then the suspension was filtered through a Whatman No. 1 filter paper using a 5 cm diameter Buchnur funnel. The crude extracts of Qat were then transferred to a 100 ml glass vial and stored as a stock solution at -20°C until used. Water Qat extracts were diluted with DW to give 2.5 and 5.0 g stock solution/Kg body weight (b. wt.).

Experimental animals: Male Swiss albino mice strain *Mus musculus demesticus*, weighing 24-31 g each, (8-12 weeks old) were used in this study. They were obtained from Zoology Department, Faculty of Science, University of Aleppo, Syria. The mice were housed (three per cage) and fed on a standard diet and water *ad libitum* and left for at least two weeks for adaptation in the laboratory environment under the conditions of 24-25°C, and relative humidity of approximately 50% and a 12:12 light dark cycle. All animals were housed in plastic cages made by London Plastic/ North Kent LTDL and given standard diet and water *ad libitum* throughout the study.

Treatment and care of animal: Male mice were separated and housed in groups for treatment (each treatment included five males). The male mice were

divided into ten groups. The first three groups were administered every other day with the same volume of distilled water and served as untreated controls. The next groups were treated orally every other day (total of 30 treatments) with the same volume of the following:

- 2.5 and 5.0 g Qat water extract /Kg (b. wt.).
- $^{1}/_{10}^{th}$ LD_{50} of dimethoate alone (14 mg/Kg body weight)
- $^{1}/_{5}^{th}$ LD_{50} of dimethoate alone (28 mg/Kg body weight)
- 2.5 and 5.0 g water Qat extract /Kg (b. wt.) + 14 mg dimethoate /Kg body weight.

The total number male mice were randomly divided into nine groups of forty-five mice each. The first group received orally 2.5 g Qat water extract /Kg body weight. The second group of animals was orally administered with 5.0 g Qat water extract /Kg body, while the third group was given $^{1}/_{10}^{th}$ LD_{50} (14 mg dimethoate / Kg body weight). The sixth group received 5.0 g Qat water extract /Kg body weight plus 14 mg dimethoate /Kg body weight and the next four groups were treated orally with the same volume of distilled water and served as untreated controls.

Recording the results: Seminal fluid analysis measurement of sperm concentration was done according to the method of Zenevld and Polokoski (1977) and Sakomato and Hashimatio (1986). Vital sperms, percentage of viable sperms were estimated according to the method of WHO (1993) and Bialy and Smith (1958). Percentage of sperm motility and sperm grade motility were recorded according to the method of Levin *et al.*, (1992).

Statistical analysis: All results were expressed as means ± S.E. values. Data were analyzed using the "t" test and analyses of variance (ANOVA) with statistical package for Social Sciences SPSS. Statistical analysis consisted of Spearman's correlation and repeated measures ANOVA followed by "t" test for paired or independent observations, as appropriate. A value of $P < .05$ was considered to be the level of statistical significance.

RESULTS AND DISCUSSION

Data presented in Table (1) clearly indicate that the percentage of sperm viability, percentage of sperm motility and sperm grade motility were not significantly affected after treatment with either 2.5 or 5.0 g Qat extract/Kg body weight compared to the control. Percentage of sperm motility, motility grade and concentration percentage were significantly affected with the higher dose of Qat extract (2.5 or 5.0g/kg b.w) compared with those untreated mice (control). The percent results are in agreement with that reported by El-Shoura (1995). They showed that heavy Qat use decreased semen volume, sperm count, and sperm motility, while it increased the number of sperm that appeared abnormal upon the microscopic exams. Interestingly, previous studies demonstrated that male rats responded similarly as men (Islam *et al.* 1990). However, other effects could only be measured in rats but cannot be ethically learned from men. Rats treated with the active constituent of Qat, cathinone, had smaller testicles, epididymides, and seminal vesicles as well as lower levels of testosterone in their blood. Microscopic examination of testicles revealed degenerative changes in cells that produce testosterone and in Sertoli cells which support sperm production in rabbits (Al-Mamary *et al.*2002). Other reports suggested that Qat stimulated spermatogenesis, and cauda epididymides while Leydig cells were found to be normal when compared with untreated rabbits. In short, there is no consensus at present regarding the possible effects

Table (1): Seminal fluid analysis in male mice treated with Qat extracts alone

Treatment	n**	% Sperm viability	% Sperm motility	Sperm grade motility	Sperm concentration ($x10^6$) / ml
		*Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Control	15	88.20a*** ± 0.73	74.20a ± 1. 31	2.72a ± 0.08	30.12a ± 0.35
2.5 g Qat extract /Kg (b.w.)	15	87.40a ± 0.59	72.13a ± 0.68	2.54a ± 0.09	29.14a ± 0.41
5.0 g Qat extract /Kg (b.w.)	15	86.80a ± 0.78	71.20b ± 0.67	2.42b ± 0.10	28.87b ± 1.13

*Data are presented as means ± S.E.

** n Three replicates, each contained 5 male mice.

***Means in a column with same letters are not-significant ($p < 0.05$) compared to the control .

of phenylpropanolamines (PPAs) on male reproductive function and that may be due to the different experimental designs employed in those studies and others.

Qureshi, *et al.*(1988) studied the cytological effects of Qat in Swiss Albino mice. They found that Qat extract significantly increased the frequency of micronucleated polychromatic erythrocytes, induced bone marrow depression and reduced the mitotic index of the somatic cells. In addition it significantly induced chromosomal aberrations viz, aneuploids, autosomal univalents, univalents of the sex chromosomes and polyploids. The frequency of abnormal sperms was also increased. Also, Qureshi *et al.*(1989) investigated the effect of Qat on the spermatogenic dysfunction in the different stages of spermatogenic cycle in mice. They observed a significant increase in sperm abnormalities in epididymal spermatozoa, late spermatids, early spermatids and spermatocytes and spermatogonia indicating stage specific sensitivity to Qat. El-Guindy (1971) demonstrated increases in temperature and pulse rate as well as mydriasis in 30 people chewing Qat. Subsequent studies described moderate increases in blood pressure, transient facial, conjunctival congestion, extra-systoles and respiratory rate. Inhibition of micurition, increased diuresis (due to intake of large amounts of fluids while chewing), increased libido, impotence and spermatorrhoea are also common (Halbach, 1979). Hammouda (1978) indicated that chloroform extract of Qat at 0.1% of diets, at different durations of treatment, decreased semen output and sperm concentration, and consequently the average of fertilized egg production. The spermatocyte division and sperm formation was markedly affected and decreased as the period of drug treatment prolonged. He also found that semen production was completely diminished after 63 days of treatment. Interestingly, testes retained normal function after withdrawal of Qat. The low production and cessation of sperms in seminiferous pathway under the effect of dietary Qat was attributed to DNA replication deficiency.

The data in Table (2) illustrated clearly that the percentage of sperm viability, percentage of sperm motility, sperm grade motility and sperm concentration were significantly ($p<0.05$) affected after treatment with $^{1}/_{10}^{th}$ LD_{50} (14 mg/kg.bw) of dimethoate alone compared to the control. It could be said that all the tested components of the seminal fluid were affected when the animals received a dose of 14 mg dimethoate /kg.bw.

Table (2): Seminal fluid analysis in male mice treated with $^{1}/_{10}^{th}$ LD_{50} of dimethoate alone.

Treatment	n**	% Sperm viability	% Sperm motility	Sperm grade motility	Sperm concentration ($x10^6$) / ml
		*Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Control	15	91.07 a*** ± 0.53	79.23 a ± 0. 61	2.60 a ± 0.11	31.83 a ± 0.35
14 mg/Kg (b. wt.) dimethoate alone	15	79.15 b ± 0.51	67.46 b ± 0.79	1.71 b ± 0.10	25.45 b ± 0.47

*Data are presented as means ± S.E.

**n Three replicates, each contained 5 male mice.

***Means in a column with same letters are not-significant ($p < 0.05$) compared to the control .

The results in Table (3) clearly indicate that the percentage of sperm viability, percentage of sperm motility, sperm grade motility and sperm concentration were significantly ($P < 0.05$) affected after the treatment with $^{1}/_{5}^{th}$ LD_{50} of dimethoate alone compared with the control. Degraeve *et al.*,(1984) conducted an experiment in which the male mice (Q. strain) received drinking water five days/week for seven weeks containing dichlorvos (2 ppm), dimethoate (0.6 ppm), malathion (8 ppm), methyl parathion (0.15 ppm) or trichlorfon (0.5 ppm). At the end of the treatment, no chromosomal damage was observed in bone marrow cells, spermatogonia and primary spermatocytes. Dominant lethal mutation assays were performed to investigate the pre- and post-implantation fetal lethality. Only negative results were obtained. Upon epididymal sperm analysis using the CASA system, all parameters including the number of sperm and sperm motions were found to be affected in those males treated with 500 mg/kg/day, and the number of sperm, percent motile, velocities and amplitude of lateral head displacement (ALH) were affected in males treated with 150 mg/kg/day. Upon sperm morphological examination, head and tail abnormalities were observed in males treated with 150 and 500 mg/kg/day. In the histopathological examination, atrophy of the seminiferous tubules and multinucleated giant cells in the testicular were observed in males treated with 500 mg/kg/day.

Farag *et al.* (2007) studied the effect of the organophosphate insecticide dimethoate at three dosage levels (7, 15, and 28 mg/kg/day) on male mice reproduction. Dimethoate was given orally by gavage to male mice for 20 days before mating with untreated females.

Table (3): Seminal fluid analysis in male mice treated with $^1/_5^{th}$ LD_{50} of dimethoate alone.

Treatment	n**	% Sperm viability	% Sperm motility	Sperm grade motility	Sperm concentration ($x10^6$) / ml
		Mean ± S.E*	Mean ± S.E	Mean ± S.E	Mean ± S.E
Control	15	86.25 a*** ± 1.10	76.91 a ± 1.08	2.41 a ± 0.09	32.24 a ± 0.32
28 mg/Kg (b. wt.) dimethoate alone	15	72.16 b ± 0.84	61.41 b ± 0.90	1.31 b ± 0.07	22.83 b ± 0.42

*Data are presented as means ± S.E.

** n Three replicates, each contained 5 male mice.

***Means in a column with same letters are not-significant ($p < 0.05$) compared to the control

Signs of cholinergic effects were observed in the 15 and 28 mg/kg/day treated groups. Brain and skeletal muscle acetylcholinesterase activities were inhibited by both middle and high doses of dimethoate. The percent morphologically normal spermatozoa were unaffected in any of the treated groups. However, sperm production and percent motile sperm were decreased in the 15 and 28 mg/kg/day treated groups compared to the control. Also, the study demonstrated the adverse effects of dimethoate on reproductive performance of male mice and pregnancy outcomes following mating with untreated female mice at dose levels of 15 and 28 mg/kg/day. The No Observed Effect Level (NOEL) for reproductive performance was 7 mg/kg/day.

As shown in Table (4) the percentage of sperm viability, percentage of sperm motility, sperm grade motility and sperm concentration were significantly ($P<0.05$) affected by the mixture of both tested Qat extracts plus dimethoate (14 mg/kg.bw). The results clearly indicate that the mixture of dimethoate /Qat extracts had more dramatic reduction in Seminal fluid than dimethoate alone, or Qat extracts.

The present study emphasized the hazard of even sublethal residues of insecticides which are in actual use on Qat plants. Such residues are of serious risk especially when no safety period after the last spray or application and before harvesting (PHI) is maintained. It is noteworthy that the tested lower level of Qat extract (2.5 g/Kg) is not an exaggerated dose, and it is in the range

of the daily intake of consumers. Besides, the $^{1}/_{10}^{th}$ LD_{50} (14 mg/kg.bw) of dimethoate is also in the range of the practical level of residues on sprayed Qat foliage especially if there is no safety period after the insecticide application and the Qat harvesting. This higher risk demonstrated in Qat leaves must give the consumers great awareness especially between youngsters to avoid the fatal habit of Qat chewing. It is also the responsibility of the health officers, educators, the media and growers to revise their philosophy and to implement a new policy in which Qat is no longer encouraged as an acceptable social habit, since it might dramatically affect the quality of animal semin.

Table (4): Seminal fluid analysis in male mice treated with Qat extracts plus $^{1}/_{10}^{th}$ LD_{50} of dimethoate

Treatment	n**	% Sperm viability	% Sperm motility	Sperm grade motility	Sperm concentration ($x10^6$) / ml
		Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
Control	15	88.76 a*** ± 0.58	76.21 a ± 0. 65	2.50 a ± 0.07	33.47 a ± 0.33
2.5 g Qat extract /Kg + 14 mg dimethoate /Kg (b. wt.)	15	82.64 b ± 0.57	66.85 b ± 0.51	1.92 b ± 0.05	29.67 b ± 0.49
5.0 g Qat extract /Kg + 14 mg dimethoate /Kg (b. wt.)	15	79.21 b ± 0.52	66.50 b ± 0.83	1.84 b ± 0.07	28.42 b ± 0.42

*Data are presented as means ± S.E.
** n Three replicates, each contained 5 male mice.
***Means in a column with same letters are not-significant ($p < 0.05$) compared to the control.

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تأثيرات مستخلصات اوراق نبات القات المرشوش بمبيد الدايمثويت على الحيوانات المنوية في ذكور الفئران

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في هذه الدراسة تم تقييم التأثيرات الضارة لمستخلصات نبات القات المعامل بالرش بمبيد الدايموثيت عند مستوى 14 مجم / كجم والمعطى عن طريق الفم وتأثير ذلك على فئرانmice كل 48 ساعة لمدة شهرين (30جرعة) مع تسجيل تأثير ذلك على خصائص الحيوانات المنوية (حركية الحيوانات المنوية - وحيويتها وتركيزها) في الحيوانات المعاملة مقارنة بغير المعاملة .

ولقد لوحظ تغيرات معنوية في حجم وعدد وحيوية الحيوانات المنوية في الافراد المعامله بالتركز 14 مجم /كجم مقارنة بالكنترول .وهكذا يتضح اخطار مستخلصات اوراق نبات القات الذى سبق معاملة وشا بالمبيدات (مثل الدايمثويت) على الصحة الانجابية للافراد الذى تعودوا على مضغ اوراق تلك المعامل بالمبيدات .وهذا ينبه اى المخاهر المحية لمثل هذه العادات التى تشكل خطورة على الانسان مما يؤكد اهمية البنية الى تلك المخاطر وضرورة ايقافها.