J. Pest Cont. & Environ. Sci. 16 (1/2): 17 – 26 (2008).

Effect of dimethoate, Qat (*Catha edulis*) and their combination on male mice body weight

Al-Arami Atieq; A. A. M. Thabet*; A. Ghrewati and A. H El-Sebae*****

Department of Zoology, Faculty of Science, Thamar University.

*Plant Protection Department, Faculty of Agriculture, Sana'a University, Republic of Yemen

** Department of Zoology, Faculty of Science, Aleppo University, Republic of Syria

***Pesticides Division, Faculty of Agriculture, Alexandria University, Chatby, Alexandria, Egypt

ABSTRACT

The protection of Qat (*Catha edulis*) from different pests is widely practiced to maintain fresh clean foliage. Dimethoate an organophosphate insecticide, is widely used on Qat trees in the Republic of Yemen and the health hazard of such insecticide is more liable with much higher risk when it is applied to edible crops which are freshly used. Therefore, the present study was aimed to evaluate both water Qat extracts alone or in combination with sublethal doses of $^{1}/_{10}^{th}$ LD_{50} or $^{1}/_{5}^{th}$ LD_{50} (14 or 28 mg/kg bw) of dimethoate on male mice for investigating their effect on body weight. There was a significant reduction in male mice body weight after treatment with 2.5 and 5.0 g Qat extract/kg bw compared with the untreated control ($P<0.05$). Simipurl the treatment with 14 or 28 mg dimethoate/kg bw showed significant reduction in male mice body weight after treatment with the higher dose ($P<0.05$), 30 days after treatment. Both tested doses of dimethoate led to decrease of – 4.12 and -7.26% compared with control animals which gained an increase ranged between 7.26-10.77% over their original weight. Also, there was a significant reduction in male mice body weight after treatment with 2.5 and 5.0 g Qat extracts /kg bw or 14 mg dimethoate / kg bw compared with untreated control ($P<0.05$), 30 days after the treatment. The higher concentration of Qat extract resulted in a decrease of body weight (– 11.53%) compared with control animals. The adverse effect evaluation of human exposure to Qat alone, or Qat contaminated with pesticide residues reveals the hazards of both acute and long-term exposure to the Qat amines that presented in Qat alone or combined with the hazardous dimethoate insecticide which show contact residual and systemic activity. Moreover, consuming Qat as fresh leaves double the risk since the farmers use this insecticide for insect control. This high risk demonstrated in Qat consumers necessitates the public 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 affects our health.

INTRODUCTION

Qat (*Catha edulis*) is considered as one of the widely consumed edible crop plants in some Arab and East African countries. Qat fresh top young leaves and shoots are the main consumed parts in the habitual chewing of Qat , under the impression of gaining a temporary stimulation. Large areas of fertile land are cultivated with Qat in many countries including the Republic of Yemen, Somalia, Ethiopia and Kenya where the climatic conditions seem to be very favorable. Qat daily consumption includes more than 5 million consumers every day worldwide. The production of Qat is now of first economic priority in many of these countries. Therefore, Qat protection from different pests is widely practiced to maintain fresh clean foliage. Therefore, Qat producers are keen to use whatever pesticides they think of for Qat plant protection. The production of Qat is now the first economic priority in many Governorates in Yemen. Qat causes various social, economic and health problems (Thabet and El-Sebae, 2008).

Pesticides are used in the developing countries and some of them still include many of the already banned or restricted insecticides such as dimethoate (an organophosphorus compound) which is restricted in its use due to its cytotoxicity potential. Pesticides which have been used on Qat trees in Yemen still include many of those banned or restricted pesticides (Thabet, 2007). Dimethoate is still widely used on Qat plants. The possibility of foliage contamination with dimethoate residues is quite expected.

The temporary stimulation effect of Qat is actually due to its content of certain amines, which are phenyl propylamine derivatives. Such compound, e.g. cathinone and cathine behave like amphetamines and are called natural amphetamines. Amphetamines are synthetic phenyl propylamine derivatives and have a spectrum of both acute and long-term side effects. Therefore, the combination of Qat amines in Qat leaves, either alone or in combination with insecticide residues, will be of concern regarding the health risks to those Qat chewers. The health hazard of such insecticides is more liable with much higher risk when they were applied on edible crops which are freshly used (Thabet, 1994).

The adverse effects on human health of Qat chewing combined with pesticides usage were investigated. Results of interviews and questionnaires showed that chewers of Qat grown with few or no chemical pesticides and chewers of Qat grown with chemical pesticides have considerably different subjective symptoms. Chewers of Qat produced in fields where chemical pesticides are used regularly have more symptoms than those chewers of Qat produced in free pesticides fields where chemical pesticides are rarely or never been used. Chewers of Qat produced with more chemical pesticides, in particular, complain of acute adverse effects on the digestive system and chronic adverse effects such as body weakness and nasal problems. Farmers who chew Qat on which they spread chemical pesticides by themselves may have the highest health risks and they concluded that chewing Qat grown with the application of chemical pesticides causes considerable adverse health effects in human beings (Junko *et al.* 2004). Al-Meshal (1987) who investigated the mutagenic potential of cathinone using dominant lethal assay procedure, in two different groups of male mice at 100 and 200 mg/kg bw. Nencini *et al.* (1988) tested cathinone as a model substance for exploring the mechanisms underlying the development of tolerance to the anorexigenic effect of amphetamine-like substances. In this context, the modification by chronic cathinone administration of the hyperphagic response to a kappa-agonistic opioid was studied in rats. It was found that pretreatment with cathinone resulted in increasing the hyperphagic response by a factor of two and that this potentiation was naloxone-reversible. This suggests that tolerance to the anorectic effect of amphetamine-like substance is associated with sensitization to kappa=opiate mediated activation of food intake.

The present investigation is dealing with the adverse effect evaluation due to exposure to Qat extracts, especially when the leaves are contaminated with insecticide residues.

MATERIALS AND METHODS

Tested pesticides: Dimethoate (40% EC), *O,O*-dimethyl S-[2-(methylamino)-2- oxoethyl] phosphorodithioate, was introduction by Cheminova Co. BAEF-Group. The acute oral LD_{50} for mice used in the present study is 140 mg/kg according to Eto (1974).

Preparation of Qat extracts: Fresh samples of stem tips and leaves of Qat, *Catha edulis* (Forskal.) were collected from Yemen fields. Five hundred grams of Qat stem tips and leaves were homogenized in 100 ml of distilled water (DW) using a Polytron homogenizer for one hour. Then, the suspension was filtered through a Whatman No. 1 filter paper using a 5 cm diameter Büchnur 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 extract was diluted with DW to give 2.5 and 5.0 g stock solution.

Experimental animal: Male Swiss albino mice strain *Mus musculus demesticus*, weighing range 24-31 each (8-12 weeks old) were used in this study. They were obtained from the 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 under laboratory conditions of 24-25°C, and relative humidity of approximately 50% and a 12 hr. light : 12 hr. dark photoperiod . 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 animals: The 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 bw. The second group of animals was orally administered with 5.0 g Qat water extract /kg bw, while the third group was given $^{1}/_{10}^{th}$ LD_{50} (14 mg dimethoate/kg bw). The fourth group was given $^{1}/_{5}^{th}$ LD_{50} (28 mg/kg bw) of dimethoate alone.The fifth group received 2.5g Qat extract /kg bw plus $^{1}/_{10}^{th}$ LD_{50} (14 mg dimethoate /kg bw). The sixth group received 5.0 g Qat water extract /kg bw plus 14 mg dimethoate /kg bw and The last group was treated orally with the same volume of distilled water and served as untreated controls.

Recording the results: Both untreated and treated male mice were kept under observation for 60 days post-treatment. Clinical signs of toxicity were recorded for each animal. Male mice body weights (MMBW) before and after treatment were recorded. The percentage of increase or decrease in the body weight was calculated using the following formula :

$$\text{Percentage of increase (+) or decrease (-)} = \frac{\text{MBWA} - \text{MBWB}}{\text{MBWB}} \times 100$$

Where: MBWA = Mean body weight after treatment.
MBWB = Mean body weight before treatment.

Statistical analysis: Results were expressed as means ± SE. Data were analyzed using paired t-test with statistical package for Social Sciences (SPSS). A value of $p<0.05$ was considered to be the level of statistical significance.

RESULTS AND DISCUSSION

Clinical signs of toxicity: Clinical signs of toxicity were observed in the treated groups. The activity of males after treatment with 2.5 and 5.0 g Qat extracts /kg bw was increased when they were compared with the control. Clinical signs in males after treatment with (14 and 28 mg dimethoate alone /kg bw) or the mixture of either 2.5 or 5.0g Qat extracts plus 14 mg dimethoate /kg bw included depression, tremors, rapid respiration and lameness of the hind limbs when compared with the control.

The effect of dimethoat, Qat extracts, and their combinations on male mice body weight:-

A. Qat Extracts: Results in Table (1) showed that there was a significant reduction in male mice body weights after treatment with 2.5 and 5.0 g Qat extract /kg body weight compared with untreated control ($P<0.05$). It was also found that as the concentration of Qat extract increases the percentage of body weight reduction was increased. Our results are in agreement with the data reported by Islam *et al.* (1990) who mentioned that cathinone enantiomers reduced the food intake, in the dose-dependent manner. Although, it has been reported that the consumption of Qat (*Catha edulis*) is an important part of social meetings in Arabian and eastern African countries, it has possible harmful effects (Imam, *et al.*, 1999). They investigated the effect of the phenylalkylamine alkaloidal fraction of *C. edulis* (0.2, 0.4, 0.8 or 2 ml doses) on the physiology of rats.

Table (1). Effect of Qat extracts on male mice body weight, 60 days post-treatment

Treatment	*n	Body weight before treatment (g)	Body weight after treatment (g)	% Increase (+) or decrease (-)
Control	15	$24.02^{a} \pm 0.08$	$25.51^{a} \pm 0.20$	+ 6.20
2.5 g Qat extract /Kg bw	15	$24.16^{a} \pm 0.26$	$21.80^{b} \pm 0.48$	- 9.77
5.0 g Qat extract /Kg bw	15	$25.06^{a} \pm 0.22$	$22.17^{b} \pm 0.37$	- 11.53

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

Also, they found that rats given the highest dose were dead. The alkaloid fraction had no effect on blood haemoglobin content. On the other hand, they observed that body weight decreased as the alkaloid dose increased.

B. Effect of dimethoate alone: The results in Table (2) clearly indicate that there was a significant redaction in male mice body weight after treatment with $^{1}/_{10}^{th}$ LD_{50} of dimethoate alone (14 mg/kg bw). The untreated mice were found to increase in body weight by about 10% of their original weight. On the other hand, the administration of 14 mg dimethoate/ kg bw, resulted in a decrease of 4.12% of body weight. The treatment with 28 mg dimethoate alone /kg bw had more dramatic reduction on male mice body weight, 60 days after the treatment. The rate of decrease in body weight increased as the concentration of the given doses increased (Table 3). This result was confirmed the results reported by Talukdar *et al.* (1985) who observed a significant decrease in body and liver weights of mice exposed to dimethoate. When mice were exposed to 60 µg/ml dimethoate in their drinking water, it affected reproduction, pup survival, and growth rates of surviving pups. Treated adults exhibited weight reduction (Meister, 1992).

Table (2). Effects of $1/10^{th}$ LD_{50} of dimethoate on male mice body weight, 60 days post-treatment

Treatment	*n	Body weight before treatment (g)	Body weight after treatment (g)	% Increase (+) or decrease (-)
Control	15	$24.14^{a} \pm 0.13$	$26.74^{a} \pm 0.19$	+ 10.77
14 mg/kg bw Dimethoate	15	$23.06^{a} \pm 0.42$	$22.11^{b} \pm 0.36$	- 4.12

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

Table (3). Effects of $1/5^{th}$ LD_{50} of dimethoate on body weight of male mice, 60 days post-treatment

Treatment	*n	Body weight before treatment (g)	Body weight after treatment (g)	% Increase(+) or decrease(-)
Control	15	$25.06^{a} \pm 0.28$	$26.88^{a} \pm 0.24$	+ 7.26
28 mg/Kg bw Dimethoate	15	$25.05^{a} \pm 0.42$	$23.23^{b} \pm 0.67$	- 7.26

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

In the present study, the influence of dimethoate as sublethal doses on the male mice illustrated marked reduction in the body weight compared to the control. The rate of decrease in body weight was increased as the concentration of the given doses increased. Sanderson and Edson (1964) maintained groups of 20 semi-adult male rats for 6 to 12 months on a diet containing various concentrations (ranging from 10-800 ppm) of dimethoate. They found that at a dose of 800 ppm, severe toxic effects (cholinergic effects, weakness, apathy and weight loss) were developed within few days and seemed likely to be lethal, so the chemical was withdrawn after one week. Recovery was completed in 10 to 14 days. Less severe toxic effects and a reduced rate of weight were induced by 200 ppm, but not by 50 ppm or below.

C. Effect of Qat extracts (2.5 or 5.0 g) plus dimethoate (14 mg/kg): The presented data in Table (4) show that there was a reduction in male mice body weight after treatment with either 2.5 or 5.0 g Qat extracts /kg plus 14 mg dimethoate /kg body weight compared with the untreated control. Due to the variation of the body weight of those animals used in this test (as control), statistical analysis was done but the percentage of increase or decrease would be useful in this case to show the effect of the different treatments. Nevertheless, both the tested treatments caused a clear reduction that ranged between 9.81 and 10.77% of the mice weight before treatments. It could be said that both treatments have more or less the same effect on those treated mice. Our results are in agreement with those of Vos and Krajnc (1983), El-Masry (1987) and El-Gendy (1991). They found that pesticides caused a marked loss of body weight as compared with control animals.

Table (4). Effects of Qat extracts combined with dimethoate on body weight of male mice, 60 days post- treatment

Treatment	*n	Body weight before treatment (g)	Body weight after treatment (g)	% Increase(+) or decrease(-)
Control	15	30.17 ^{a} ± 0.22	31.80 ^{a} ± 0.22	+ 5.40
2.5 g Qat extract /kg + 14 mg dimethoate /kg bw	15	28.32 ^{a} ± 0.25	25.27 ^{b} ± 0.70	- 10.77
5.0 g Qat extract /kg + 14 mg dimethoate /kg bw	15	31.10 ^{a} ± 0.26	28.05 ^{b} ± 0.25	- 9.81

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

The present data supports the findings that cathinone produced a dose-dependent decrease in food consumption and suppressed the body weight of mice. The present study also emphasized the hazard of even sublethal residues of insecticides which are in actual use and applied on Qat plants. Such residues are of serious risk especially when no safety period after the last spray or application and before harvesting (PHI = Pre Harvest Interval) is maintained. Combination of residues of pesticides with Qat is one of the sources of additional hazards to Qat consumers as investigated in this study. Therefore, the hazards of Qat are expected to be intensified in the presence of insecticide residues.

The advance effect resulted from human exposure to Qat alone, or Qat contaminated with pesticide residues reveals the hazards of both acute and long-term exposure to the Qat amines in Qat alone or combined with the hazardous insecticides such as the systemic insecticide dimethoate.

REFERENCES

Al-Meshal, I.A. (1987). Studies on the preparation of an active constituent of Qat *Catha edulis* and its mutagenic activity in mice. Res. Commun. Subst. Abuse, 8: 49-58.

El-Gendy, K.S. (1991). Biochemical targets affected by sublethal doses of lindane and deltamethrin. J. Pest. Cont. Environ. Sci., 3 (2): 63-76.

El-Masry, S.R.D. (1987). Immunological, biochemical and histopathological studies of two commonly used hepatotoxic pesticides. Ph.D. Thesis, Medical Research Institute, Alexandria Univ., Egypt.

Eto, M. (1974). Organophosphorus pesticides: Organic and biological chemistry. CRC Press Inc.: Cleveland, Ohio, pp. 387.

Imam, S.A.; Heikal, O.A.; Mahran, L.G; Diab, A.M. and Shata, W.A.L. (1999). Toxicological effects of the phenylalkylamines alkaloidal fraction of Qat (*Catha edulis,* Forsk) extract on body weight, blood picture and liver cells of albino rats. Bull. Nat. Res. Centre, 24 (1): 41-58.

Islam, M.W.; Tariq, M.; Ageel, A.M.; Foraly, F.S. and Al-Meshal, I.A. (1990). An evaluation of the male reproductive toxicity of cathinone. Toxicol., 60: 223-234.

Junko, D; Noritoshi, T. and Tatsuya, H. (2004). Qat chewing and pesticides: a study of adverse health effects in people of the mountainous areas of Yemen. Inter. J. Environ. Health Res., 14(6): 405- 414.

Meister, R.T. (ed.). (1992). Farm Chemicals Handbook '92. Meister Publishing Company, Willoughby, OH, U.S.A.

Nencini, P.; Johanson, C. and Schuster, C. (1988). Sensitization to kappa opiod mechanisms associated with tolerance to the anorectic effects of cathinone. J. Pharmac. Exp. Ther., 245: 147-154.

Sanderson, D.M. and Edson, E.F. (1964).Toxicological properties of the organophosphorus insecticide dimethoate. Br. J. Ind. Med., 21: 52 .

Talukdar, A. R.; Sharma, A. and Talukar, G. (1985) Cytotoxic effects of dimethoate. Nucleus, 28 (3): 243-259.

Thabet, A.A.M. (1994). Interaction between the extract of a natural plant and toxicology of some insecticides. Ph.D. Thesis, Faculty of Agriculture, Alexandria University, El-Chatby, Alexandria, Egypt.

Thabet, A.A.M. (2007). List of pesticides banned and severely restricted in Yemen Republic Ministry of Agriculture & Irrigation. General Directorate of Plant Protection, 1-27.

Thabet, A.A.M and El-Sebae, A.H. (2008). Survey of the type and intensity of pesticides used on Qat trees in Yemen and their adverse health impact . In Press.

Vos, J.G. and Krajnc, E.I. (1983). Immunotoxicity of pesticides. Toxicol. Environ. Sci., 11: 229.

تأثير مستخلص القات ومخاليطها مع مبيد الديمثويت على أوزان ذكور الفئران

عتيق العرامي - *عبد الرحمن ثابت - **عبد الجليل غريواتي-* عبد الخالق السباعي**
قسم الحيوان - كلية العلوم - جامعة ذمار -الجمهورية اليمنية
*قسم وقاية النبات - كلية الزراعة - جامعة صنعاء- الجمهورية اليمنية
** قسم الحيوان - كلية العلوم - جامعة حلب- الجمهورية السورية
*** قسم كيمياء المبيدات - كلية الزراعة - جامعة الإسكندرية- جمهورية مصر العربية

الأهمية الاقتصادية لشجرة القات تعتمد على النموات الحديثة للفروع والأوراق التي تتعرض للإصابة بالآفات الحشرية لذلك يحرص المزارعون على استخدام المبيدات الكيميائية بهدف الحصول على وفرة من أغصان القات. والخطر الكبير الذي يمثله القات يكمن في متبقيات المبيدات في عصارة القات خاصة من المبيدات الجهازية. وكون القات شجرة مربحة نجد المزارع شديد الحرص على المحافظة عليها. لذلك يستخدم خليط من المبيدات الكيميائية حيث وصل المزارع في استخدامه العشوائي للمبيدات إلى مستويات خطرة جداً على صحته والبيئة التي يعيش فيها بالإضافة إلى مستهلكي القات خاصة أن القات يؤكل طازجاً. الأمر الذي يستدعي دراسة تأثير القات ومتبقيات المبيدات على صحة الإنسان .

وقد أظهرت النتائج حدوث انخفاض معنوي في معدل أوزان أجسام الفئران المعاملة بمستخلص القات للتركيزين 2,5 جرام و 5 جرام / كيلوجرام من وزن الجسم عند المقارنة قبل و بعد المعاملة . كما ظهر انخفاض معنوي في معدلات أوزان أجسام الحيوانات للمجموعة التجريبية المعاملة بـ 14 و 28 ملليجرام دايمثويت / كيلوجرام من وزن جسم الحيوان. كما أظهرت النتائج حدوث انخفاض معنوي في معدل أوزان أجسام الفئران المعاملة بمستخلص القات (2,5 أو 5 جم مخلوطا مع 14 مجم/كجم من وزن الجسم من مبيد الدايمثويت.