J. Agric. Sci. Mansoura Univ., 31 (9): 5809 - 5819, 2006

PROTECTIVE EFFECT OF MOLOKHIA (*Corchorus olitorius* L.) ON MALATHION-INDUCED HEPATOTOXICITY IN RATS

Zohair, Azza
Faculty of Specific Education, Home Economics Science Department, Menufiya University, Ashmoun, Menufiya, Egypt.

ABSTRACT

Molokhia (Corchorius olitorius) is a common edible and famous vegetable in Egypt has high nutritive value as well as antioxidant compounds. Therefore the protective effects of molokhia against organophosphorous pesticide (malathion) - induced hepatotoxicity in rats were studied. The results revealed that administration of malathion (20ppm) for 4 weeks, significantly depleted glutathione content (GSH), total protein (TP) and albumin, elevated glutathione-S-transferase (GST), alanine aminotranferase (ALT), aspartate aminotranferase (AST) and alkaline phosphatase (ALP) activities ($P<0.05$).However, the intake of molokhia (100mg/kg body weight) alone or molokhia supplemented to malathion treated group significantly alleviated GSH, TP and albumin depletion and the elevation of GST, ALT, AST and ALP activities ($P<0.05$). These results indicate malathion- induced hepatotoxicity and highlight the protective action of molokhia as a potential protective agent against malathion induced hepatotoxicity, suggesting that molokhia contains specific active compound(s) or the considerable amounts of general compo:nds possessing the antagonistic (phenolic c)mpounds, vitamin A, Vitamin C and chlorophyll).

Keywords: Organophosphate pesticide, Malathion, Molokhia, Corchorus olitorius, liver.

INTRODUCTION

Malathion (o,o-dimethyl s-1,2 bis-ethoxy carbonyl ethyl phosphodithiolate), is one of the most widely used organophosphate insecticides through the world. It is used to control pests affecting agricultural crops, ornamentals, greenhouses, livestock, stored grain, forests, buildings, households, gardens. Additionally, malathion is used to kill mosquitoes and to treat head lice on human. Contributing to its popularity it is relatively low acute mammalian toxicity (Brenner, 1992; Hazarika *et al.*, 2003). However, like other pesticides that have been found to cause serious health hazards, malathion may pose a greater risk than the product label which would one to believe. It has been shown that, malathion affect the central nervous system, immune system, adrenal glands, liver, blood (ATSDR, 2003), disturb the biochemical and physiological functions of erythrocytes and lymphocytes (Banerjee *et al.*, 1999) and induced oxidative stress leading to generation of free radicals and altemation in oxidants scavenging enzymes system (Banerjee *et al.*, 1998; Ahmed *et al.*, 2000; Timur *et al.*, 2003). Exposure to malathion can occur through food, water, air, soil, or surfaces such as playground equipment or pavements; the effects of exposure depend on the dose, the duration, personal traits and habits, and whether other chemicals are present (EPA, 2005). The Food and Drug Administration (FDA) and the Environmental protection Agency (EPA) allow a maximum amount of 8ppm of malathion to be present as a residue on specific crops used as foods, and 0.1mg/L to be present in drinking water for lifetime exposure of adults.

Due to the large scale proliferation of environmental pollution, search for agents capable of minimizing their toxicity on human health become very essential. Recently, there is considerable emphasis on identifying the potentisal of natural plant products as chemopreventive agents present in food consumed by human population (Wanger, 1990; Kelloff *et al.*, 1994; Ahmed *et al.*, 2000). Molokhia (Corchorus olitorius) is a common edible and famous vegetable in Egypt. It has a very high nutritive value as well as active compounds. Innami *et al.*, (1995) found that the leaf powder of molokhia and its water-souble viscous solution led to decrease total serum and liver cholesterol and increased fecal extraction of bile acid, total neutral sterols and cholesterol. It was also found that molokhia leaves suppress elevation of postprandial blood glucose levels in rats and humans (Innami *et al.*, 2005). Three cardenolides were isolated from molokhia seed-leaves and their cytotoxic activities were evaluated against six cancer cell lines as found by Abdel Wahab *et al.*, (1999). Flavonoid is one of the possible candidates of the active compounds in molokhia. It abundantly contains 5-caffeoylquinic acid, 3,5-dicaffeoylquinic acid, quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-(6-malonylglucoside), quercetin 3-(malonylgalactoside), ascorbic acid, α-tocopherol, and chlorophyll, and the content of quercetin glycosides is remarkable (Azuma *et al.*, 1999). Molokhia specially suppressed aryl hydrocarbon receptor, induced by dioxins, suggesting that molokhia contains the unique or specific active compound(s) or the considerable amounts of general compounds possessing the antagonistic action (Nishiumi *et al.*, 2006).

Therefore, it is highly possible that molokhia could also protect liver against malathion-induced toxicity via preventing or alleviating intracellular GSH depletion and oxidation damage. In view of this the purpose of study was to evaluate the in vivo protection from molokhia in malathion treated rate.

MATERIALS AND METHODS

Chemicals

All chemicals used in this study were obtained from Sigma Chemical Company (St. Louis, USA). Commerical kits were purchased from BioMerieux Company (L'Etoile/France) and from Eagle Diagnostics (Dollas, TX, USA).

Animals

Three to four-weeks old male albino rats, were obtained from the Animal House Colony, Giza, Egypt. Rats were housed on a 12h light-12h dark schedule, and fed with water ad-libitum, and rat standard diet containing by weight (g/100g): 64 starch, 23 protein, 3.5 fat, 5 fiber, 1 vitamin mixture and 3 salt mixture as mentioned by National Research Council (NRC, 1978).

Preparation of molokhia supplemented diet

Molokhia (Corchorus oliorius L.) leaves were purchased from the local market, pulverized and lyophilized using freez dryer system (Dura-Dry Freeze Dryer, Model PAC-TC-V4; FTS system, Inc., Stone Ridge, NY. USA). The dried molokhia was stored in a freezer until used.

Chemical analysis of molokhia

Moisture content, protein, fat, carbohydrate, fiber and ash were determined according to AOAC (1990). Vitamin A and C were determined as described by Strong and Koch (1976). Total free phenols were measured using the Folin-Denis reagent as described by Swain and Hillis (1959). Chlorophyll was determined according to Wettestein (1957).

Experimental design

Malathion containing diet was prepared and standardized as described by Banerjee and Hussain (1986). In brief 20mg malathion was mixed in 30ml groundnut oil to produce feed containing 20ppm malathion. This was incorporated in 1 kg diet and thoroughly mixed to ensure even distribution. The rats were randomly divided into four groups of 10 animals each and treated for 4 weeks as follows: Group I-control: rats fed on normal diet, Group II-molokhia: rats fed on 100gm/kg bw molokhia, Group III-malathion: rats received 20ppm malathion along with normal diet and Group IV-malation +molokhia: rats received 20ppm malathion along with 100mg/kg bw molokhia. Food consumption, general condition and any other symptoms were observed daily and body weights were recorded weekly.

Samples

At the end of the experimental period, fasting blood samples were collected from the retro-orbital sinus from all animals under ether anesthesia. Blood samples were left to clot and centrifuged at 5000g while cooling (5C) for 10 min to separate the serum. The clear serum was kept at -20C until analysis. The effect of malathion and molokhia on liver was assayed by assayed serum transaminases, alanine aminotranferase (ALT) and aspartate aminotranferase (AST) activites according to the method of Reitman and Frankel (1957); alkaline phosphatase (ALP) by the method of Roy (1970); colorimetric determination of albumin using bromocresol green at PH 4.2 according to the method of Doumas *et al.*, (1971); estimation of total protein (TP) according to the method described by Gornall *et al.*, (1949),by using commercial test kits obtained from BioMerieux Company (L'Etoile/France). Glutathione (GSH) level activity in serum was measured by the method of Paglia and Valentine (1967) and serum glutathione-S-transferase (GST) was measured by the method of Habig *et al.*, (1974), using commercial kits purchased from Eagle Diagnostics (Dollas, TX, USA).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and computing using the SAS General Linear Model producer (SAS, 1990). Differences with $P< 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

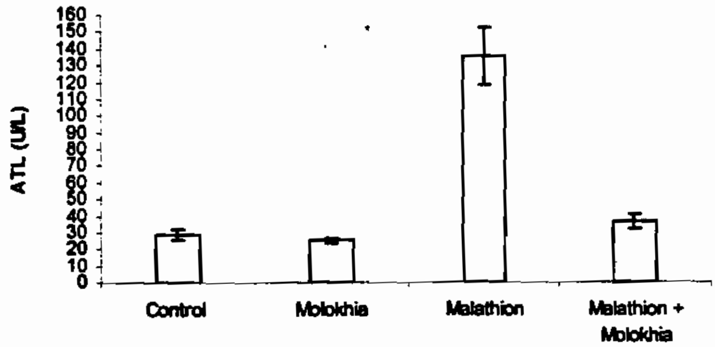
The liver is one of the major organs for detoxification of xenobiotics. At present there is considerable interest in free radical mediated damage to biological systems due to pesticide exposure (Ahmed *et al.*, 2000). A large number of xenobiotics have been identified to have potential to generate free radicals in biological system (Kehrer, 1993). Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics. Standard hematological and serum chemistry panel tests have been widely used in clinics to monitor the adverse effects resulting from diseases or exposure to xenobiotics (Abou Zeid *et al.*, 1993). A study of some commonly used plant as antioxidants against xenobiotic therefore appeared to be of interest.

The results in Fig 1 illustrate the effect of different treatments on serum enzymes ALT, AST and ALP which is known as liver functions. Malathion treatment caused significant increase in ALT, AST and ALP activities ($P<0.05$) in rats fed the normal diet. However, the level of enzymes was significantly lower in rats fed molokhia supplemented diet as compared to control or malathion-treated group. The intakes of molokhia significantly alleviate the elevation of enzymes activities ($P<0.05$) in malathion-treated rats. The increased level of ALT, AST and ALP activites may indicate degenerative change and hypofunction of liver. In clinical diagnosis, increased in these enzymes indicates affected liver (Hsu *et al.*, 2006). The affected liver function by malathion is typically to those reported by Jabbar *et al.*, (1990) who found that short term (24h) and long term (4 weeks) of malathion-treated rats increased activities of ALT, AST and ALP.

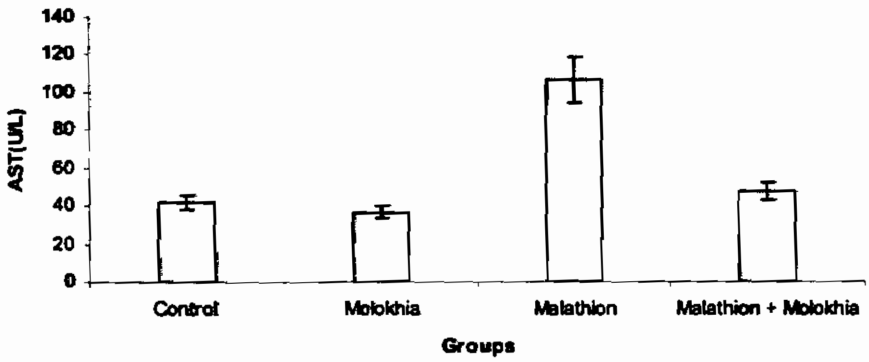
As reported by others (Abou Zeid *et al.*, 1993; Hazarika *et al.*, 2003; Timur *et al.*, 2003), malathion induced the level of TP and albumin in the rats serum. This study confirmed those previous studied. Serum TP and albumin were significantly decrease in animals treated with malathion ($P<0.05$), whereas animals treated molokhia alone or molokhia supplemented to malathion treated group show a significant improvement in both parameters (Fig 2). The reduced levels of TP and albumin may be due to inhibition of tRNA-synthetase accompanied with a lower protein synthesis and /or protein catabolism accompanied in impaired production of functional protein in the organs, in liver affected by chemical compounds, and in protein losses from the organism, increased blood vessel permeability and affected kidney (Robert *et al.*, 1993). Takeo (1987) emphasized that human albumin has a significant high affinity in binding with alkylating agent and xenobiotics. Hence, it can be anticipated that human serum albumin and its other protein will be more susceptible and highly affected by the exposure to acute poisoning pesticide such as malathion (Abou Zeid *et al.*, 1993).

As shown in Fig (3) malathion depleted GSH and increased GST activities. The decrease in GSH and increase in GST due to malathion treatment was significantly reduced ($P<0.05$) when diet supplemented with molokhia. The decrease in GSH content after malathion exposure was related to utilization of antioxidant in the detoxification of this pesticide through GST. The depletion of GSH and increased GST after malathion exposure was observed by (Ahmed *et al.*, 2000; Timur *et al.*, 2003). The GSH depletion, especially occurred in acute hypotoxicity necrosis, liver failure or death (Hsu *et al.*, 2006). However the intake of molokhia alleviated malathion-induced depletion of GSH content and consequently reduced damage in liver.

ALT



AST



ALP

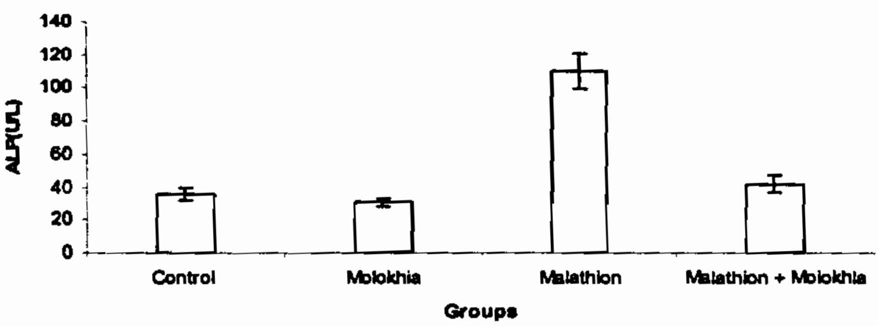


Fig . 1. Effect of Molokhia on serum ALT, ATS and ALP in rats fed malathion contaminated diet.

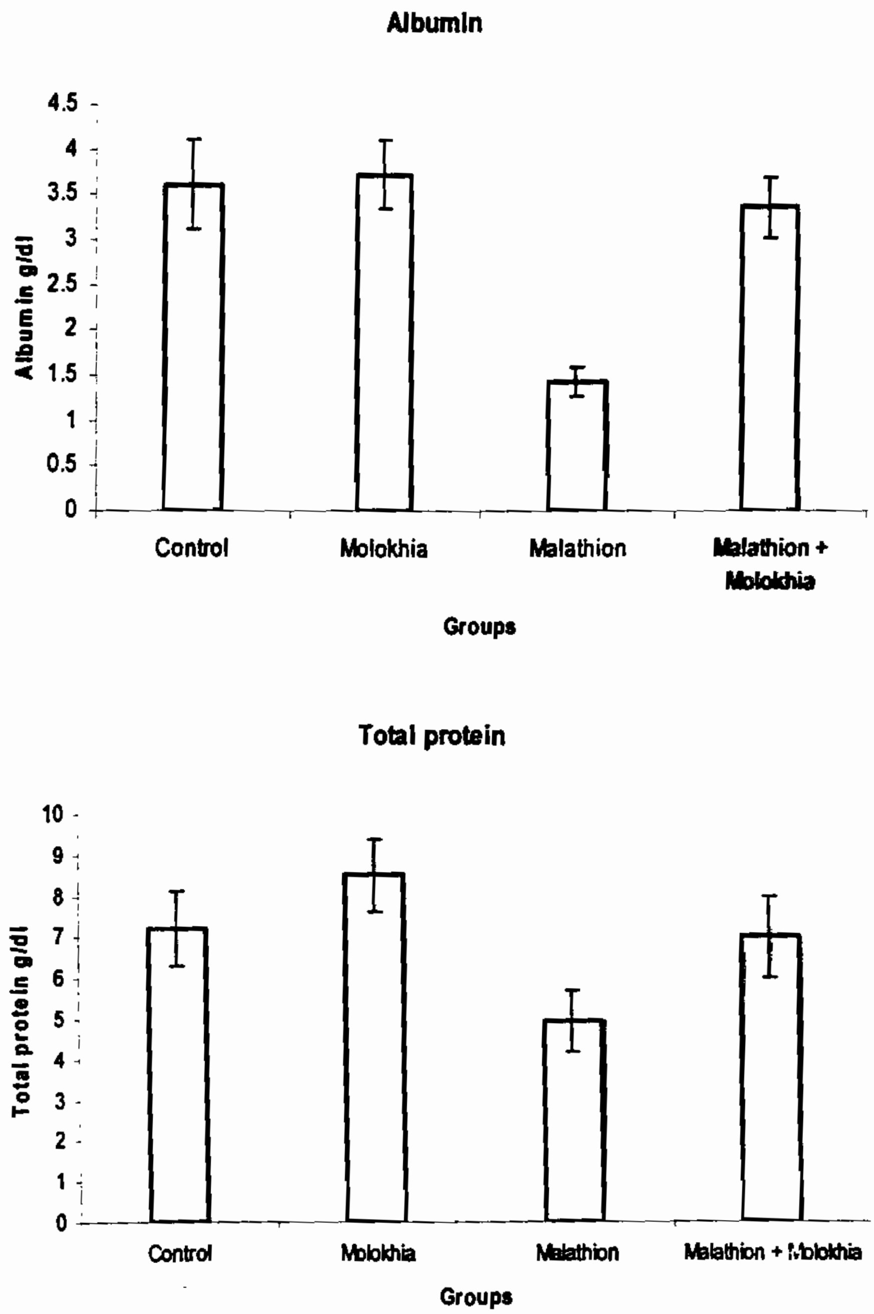
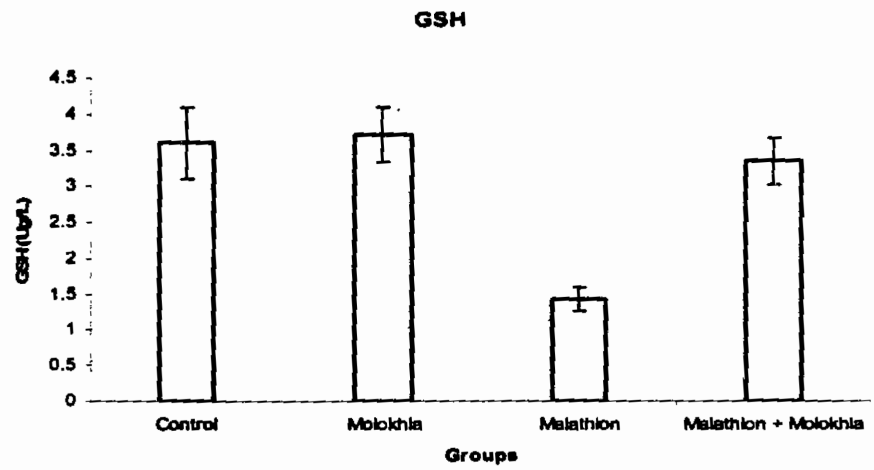


Fig. 2. Effect of Molokhia on serum total protein and albumin in rats fed malathion contaminated diet.



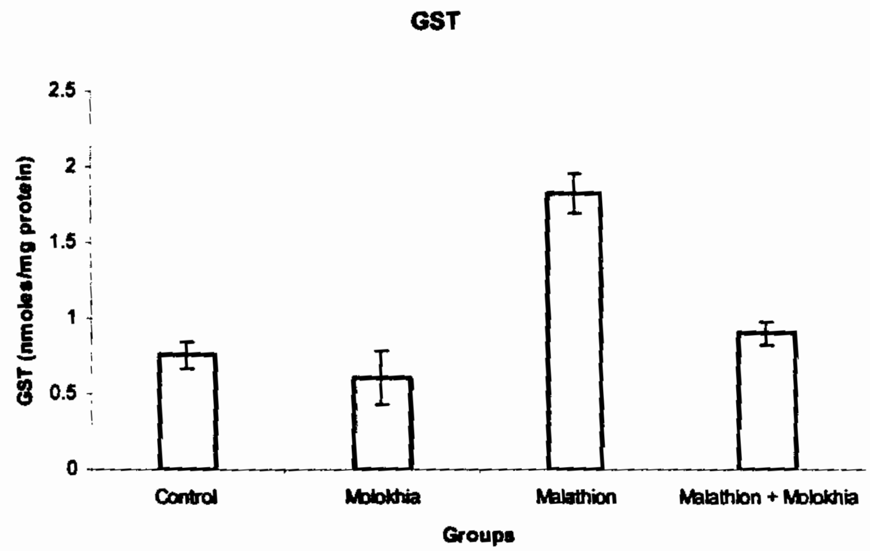


Fig. 3. Effect of Molokhia on serum GSH and GST in rats fed malathion contaminated diet.

Dietary supplemention of molokhia alleviated GSH depletion and improved liver functions in malathion-treated rats, suggesting that molokhia contains specific active compound(s) or the considerable amounts of general compounds possessing the antagonistic action. The chemical composition of molokhia (Table 1) revealed that fresh and freeze dried molokhia leaves contained high nutritive value, chlorophyll as well as high amounts of active compounds (phenolic compounds and vitamins). Phenolics are known as potential chemopreventive agents (Urso and Clarkson, 2003). Six phenolic antioxidant compounds in molokhia were identified by Azuma *et al.*, (1999) and it was found that quercetin glycoside was the predominant phenolic antioxidant. Another candidate for the active compounds in molokhia is vitamin A and C. Ascorbic acid (vitamin C) widely known as antioxidant, interacts with free radicals and oxidative product to protect cells against the genotoxicity of various oxidants (Urso and Clarkson, 2003). Another important compound is vitamin A. it is antioxidant and free radical scavenger, when supplements to the diet diminish symptoms of organochlorine pesticide DDT (Calabrese,1980). Three cardenolids were isolated from molokhia and showed cytotoxic activities against six cancer lines (Abdel Wahab *et al.*, 1999).

Table: 1. Chemical composition of Molokhia.

Chemical composition	Fresh	Freeze dried
Moisture%	80.15±6.0	5.21±0.26
Protein%	6.63±0.39	30.20±2.71
Fat%	0.30±0.01	1.94±0.09
Fiber%	7.50 ± 0.42	33.52±2.52
Ash%	3.18 ±0.16	13.90±0.69
Carbohydrate%	2.39±0.14	15.29±0.78
Vitamin A (IU)	9500±1357	9500±1350
Vitamin C (mg/100g)	60.42 ±4.0	59.80±3.50
Total soluble phenolic compounds%	4.11± 0.2	4.0±0.27
Total chlorophyll mg/100g	488.57±21.68	487.16±22.51

The other mechanism by which molokhia suppressed the toxicity of malathion may be attributed to the interaction of chlorophylls and fibers with malathion and inhibit absorption of malathion from intestine. Fukuda *et al.*, (2004) found that chlorophyll suppressed transformation of the aryl hydrocarbon receptor induced by dioxin. On the other hand Nishiumi *et al.*, (2006) found that molokhia extract administered rat liver revealed a tolerance to dioxin-induced aryl hydrocarbon transformation, suggesting that molokhia contains two classes of active compounds; the first class comprises direct inhibitor(s), which is able to permeate the intestinal cell and /or hepatocytes, and the second is the latent active compound(s), which reveals the suppressive effect after permeation and metabolic conversion in the intestinal cells and /or hepatocytes.

In conclusion, this study revealed that molokhia is a potential multiple-protective against malathion-induced hepatotoxicity and it is an attractive food for isolation and identification of a natural antagonist for pollutants and human diseases.

REFERENCES

Abdel Wahab, S.M.; Islam, W.T.; El-Tanbouly, N.D., 1999. cardenolides from Corchorus olitorius L. seed-leaves. Bulletin Faculty of Pharmacy, Cairo University 37, 149.

Abou Zeid, M.; El-Barouty, G.E.; Abdel-Reheim, E.J.; Blancato, J. Dary, C.; El-Sabae, A.H.; Saleh, M.A., 1993. Malathion disposition in dermally and orally treated rats and its impact on the blood serum acetylcholine esterase and protein profile. J. Environ. Sci. Health B28, 413-430.

Ahmed R.S.; Seth, V.; Pasha, S.T.; Banerjee, B.D., 2000. influence of Dietary Ginger (Zingiber officinales Rosc) on oxidative stress induced by malathion in rats. Food and Chemical Toxicology 38, 443-450.

ATSDR Agency of Toxic Substances and Disease Registry 1003. Toxicology profile for malathion. Atlanta, GA: U.S. department of Health and Human Services, Public Health Service.

A.O.A.C. 1990. Association of Official Analytical Chemists. Official methods of analysis. Washington, D.C. 15th Ed.

Azuma, K.; Nakayama, M.; Koshioka, M.; Ippoushi, K.; Yamaguchi, Y.; Kohara, K.; Yamauchi,Y.; Ito, H.; Higashio, H., 1999. Phenolic antioxidants from the leaves of Corchorus olitorius L. J. of Agric and Food Chemistry 47, 3963- 3966.

Banerjee, B.D.; Hussain, Q.Z., 1986. Effect of sub-chronic endosulfan exposure on humeral and cell mediated immune responses in albino rats. Archives of Toxicology 59, 279-284.

Banerjee, B.D.; Seth, V.; Pasha, S.T.; Chakraborty, A., 1998. malathion induced oxidative stress and immune suppression in rats. Immunologist, ٩, 496.

Banerjee, B.D.; Seth, V.; Bhattacharya, A.; Chakraborty, A.K., 1999. Biochemical effects of some pesticides on the lipid peroxidation and free radical scavengers. Toxicology letters 107, 33-47.

Brenner, L.1992. Malathion Fact Sheet. Journal of Pesticide Reform, Northwest Coalition for Alternatives to Pesticides, Eugene, OR.12:1-17.

Calabrese EJ. 1980. Nutrition and Environmental Health. The Influence of Nutritional Status of Pollutant Toxicity and Carcinogenicity. Vol 1, the Vitamins. New York: John Wiley &Sons, p.61.

Doumas, B.T.; Watson, W.A.; Watson, W.A.1971. Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chem Acta 31, 87-96.

EPA, Environmental Protection Agency, 2005. Malathion revised risk. Assessement Fact Sheet, September 30.

Fukuda, I.; Sakane, I.; Yabushita, Y.; Kodoi, R.; Nishiumi, S.; Kakuda, T.; Sawamura, S; Kanazawa, K.; Ashida, H., 2004. pigments in green tea leaves (Camellia sinensis) suppress transforamationof the aryl hydrocarbon receptor induced by dioxin. J. of Agric and Food Chemistry 52, 2499-2506.

Gornall, A.G.; Bardawill, C.J.; David, M.M., 1949. determination of serum proteins by means of the Biuret reaction. J. Biol. Chem. 177, 75-79.

Habig, W.H.; Pabst, M.J.; Jacoby, W.B. 1974. glutathione-S-transferase: the first step in mercapturic acid formation. J. of Biological Chemistry 249, 7130-7139.

Hazarika, A.; Sarkar, S.N.; Hajare, S., Kataria, M.; Malik, M.K., 2003. influence of malathion pretreatment on the toxicity of anilofos in male rats: a biochemical interaction study. Toxicology 185, 1-8.

Hsu, C.C.; Lin, C.C.; Liao, T.S.; Yin, M.C., 2006. protective effect of s-allyl cysteine and s-propyl cycteine on acetaminophen-induced hepatotoxicity in mice. Food and Chemical Toxicology 44, 393-397.

Innami, S.; Nakamura, K.; Tabata, K.; Woda, M.; Takita, T., 1995. water soluble and viscous substances of Jew's mallow leaves lower serum and liver cholesterol concentration and increase fecal steroid excretion in rats fed on rich cholesterol diet. J. of Nutrition Science and Vitaminology 41, 465.

Innami, S.; Ishida, H.; Nakamura, K.; Kondo, M.; Tabata, K.; Koquchi, T.; Shimizu, J.; furusho, T., 2005. Jew's mellow leaves (Corchorus olitorius) suppress elevation of postprandial blood glucose levels in rats and humans. Int J Vitam Nutr Res 75, 39-46.

Jabbar, A.; Khawaja, S.A.; Iqbal, A.; Malik, S.A., 1990. Effect of malathion and methyl-parathion on rat liver enzymes.J Pak Med Assoc 40,266-270.

Kehrer, J.P. 1993. Free radical as mediator of tissue injury and disease. Critical Reviews in Toxicology 23, 21-48.

Kelloff, G.J.; Boone, C.W.; Growell, J.A.; Steele, V.E.; Lubit, R.; Sigman, C.C., 1994. chemopreventive drug development: perspectives and progress. Cancer Epidemiology biomarker and Preventation 3, 85-98.

National Research Council(NRC),1978.Nutrient Requirements of laboratory Animals, National Academy of Sciences 3rd ed.

Nishiumi, S.; Yabushita, Y.; Fukuda, I; Mukai, R; Yoshida, K; Ashida, H, 2006. Molokhia (Corchorus olitorius L.) extract suppresses transformation of the aryl hydrocarbon receptor induced by dioxins. Food and Chemical Toxicology 44, 250-260.

Paglia, D.E.; Valentine, W.N.1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab. Clin. Med. 70, 158-169.

Reitman, S.; Frankel, S., 1957. Colorimetric method for aspartate and alanine transferase. Am. J. Clin. Pathol. 28, 56-63.

Robert, K.M, Daryl, K.G.; Peter, A.M and Victor W.R.1993. Harper Biochemistry 23ed. USA.

Roy, A.V., 1970. Rapid method for determing alkaline phosphatase activity in serum with thymolphthalin monophosphate. J. Clin.Chem. 16, 431-436.

SAS/STAT User's Guide,1990.Statistical Analysis System institute,Cary,NC.

Strong, F.M; Kock, G.H., 1976. Colorimetric determination of vitamin A and C. Biockemistry (Laboratory manual) 2nd ed. WMC. Brown Company Publishers, Dubuque, USA.

Swain, T.; Hillis, A.E., 1959. The quantitative analysis of ohenolic constituent. J. Sci. Food Agric. 10, 65.

Takeo, K., 1987. "Affinity electrophrophoresis" In: Advancedelectrophoresis, Edit. Chrambach, A; M.J. Dunn and B.J. Radola., vol. 1 VCH Publisher, Germany.

Timur, S.; Onal,S.; Karabay, N.U.; Sayim, F.; Zihnioglu, F., 2003. In vivo effects of malathion on glutathione -s- transferase and acetylcholineesterase activities in various tissues of neonatal rats. Turk J Zool 27, 247-252.

Urso, M.L.; Clarkson, P.M., 2003. Oxidative stress, exercise and antioxidant supplementation. Toxicology 189, 41-54.
Wanger, H., 1990. Search for plant derived natural products with immunostimulatory activity (resent results).Pure and Applied Chemistry 62 1217-1222.
Wettestein, D.V., 1957. Chlorophyll-letate and dersubmikroskopischer from wackee derplastischen. Experimental cell Research 2, 427.

دور الملوخية الوقائي للكبد ضد السمية الناتجة عن مبيد المالثيون

عزة زهير
قسم الأقتصاد المنزلي كلية التربية النوعية – جامعة المنوفية

يعتبر المالثيون من أكثر المبيدات الفوسفورية شائعة الأستخدام في جميع أنحاء العالم بهدف حماية المنتجات الزراعية ، ونباتات الزينة والحبوب المخزنة كما يستخدم في المباني والمنازل والحدائق للوقاية من الآفات بالأضافة إلي استخدامه لقتل الباعوض والقمل . وبذلك نجد أن الأنسان يتعرض لهذا المبيد بطرق مختلفة نتيجة تواجده في الماء والغذاء وكل ماهو محيط بالأنسان . والمالثون مثل أغلب المبيدات لـه أثار خطيرة علي الصحة العامة وتكمن خطورته في أثاره الضارة علي الجهاز العصبي المركزي والجهاز المناعي حيث يعتبر المالثيون من المواد الكيميائية التي تنتج الشوادر الحرة (free radicals) والتي تعرف بأنها الجزيئات المحتوية علي اليكترونات غير مزدوجة في مدارها الخارجي مما يجعلها شرهة للتفاعل ومن ثم تهاجم الجدار الخلوي لسحب الأليكترونات منه لأستكمال المدار الخارجي لها والذي يؤدي بدوره إلي تدمير الخلايا .

لذا كان ممن الضروري البحث عن مواد لها القدرة علي الحد من أو منع التأثيرات السامة والضارة لهذه الملوثات الكيميائية. وقد أتجهت الأنظار في الآونة الأخيرة إلي بعض النباتات التي يتناولها الأنسان في غذائه وتتميز بأحتوائها علي مواد فعالة لها القدرة علي حجز الشقوق الحرة التي تنتج من المبيدات وبالتالي الحد من تأثيراتها الضارة علي صحة الأنسان.

لذلك كان الهدف الأساسي من هذه الدراسة هو تقييم التأثير الواقي المتمثل في مضادات الأكسدة الموجودة في نبات الملوخية ضد التأثيرات الضارة لمبيد المالثيون علي الكبد. وقد تمت الدراسة بأجراء التجارب علي مدار أربعة أسابيع علي عدد أربعون فأر ذكر من فئران التجارب التي تم تقسيمها إلي أربعة مجموعات المجموعة الأولي الضابطة (الكونترول) والمجموعة الثانية تم تغذيتها علي عليقة تحتوي علي الملوخية المجفدة بنسبة ١٠٠ ملجم ملوخية مجفدة لكل كجم من وزن الفأروالمجموعة الثالثة وتم معاملة العليقة لها بالمالثيون بنسبة ٢٠ ملجم من المبيد لكل كجم من العليقة ثم المجموعة الرابعة والأخيرة وتم معاملة العليقة فيها بالمبيد بنفس النسبة السابق ذكرها في المجموعة الثالثة مع أضافة ١٠٠ ملجم من الملوخية المجفدة لكل كجم من وزن الفأر إلي العليقة.

وقد أوضحت نتائج الدراسة أن للمالثيون تأثير سام علي الكبد حيث أدي إلي زيادة ملحوظة ($P<0.05$) في مستوي الألانين أمينوترانسفيريز (ALT) وأسبارتات أمينوترانسفيريز (AST) وكذلك زيادة ملحوظة ($P<0.05$) في مستوي الألكالين فوسفاتيز (ALP) والجلوتاثيون -أس-ترانسفيريز (GST) . بينما كان هناك أنخفاض ملحوظا ($P<0.05$) في كل من الألبيومين والبروتين الكلي والجلوتاثيون الكلي . وعلي الجانب الأخر نجد أن المجموعتان اللتان تغذيتا علي عليقة مضافا اليها الملوخية فقط أو الملوخية والمالثيون كان هناك تحسن معنوي ملحوظا ($P<0.05$) في جميع القياسات السابقة . وهذه النتيجة تؤكد مدي أهمية تناول الملوخية كغذاء واقي للكبد ضد التأثير السام لمبيد المالثيون وذلك يرجع إلي أحتواء الملوخية علي العديد من مضادات الأكسدة مثل فيتامين أ ، ج و الفينولات التي لها القدرة علي ربط الشوادر الحرة الناتجة عن المبيد، أو ربما يحدث هذا التأثير الواقي من خلال الكلوروفيل والألياف الموجوده بالملوخية حيث تتفاعل مع المالثيون وبالتالي تثبيط أمتصاص المبيد خلال الأمعاء .

ونستخلص مما سبق أن الملوخية غذاء ممتاز له العديد من التأثيرات الواقية والمفيدة للكبد ضد التسمم الناتج عن المالثيون وكذلك يمكن أن نعزل منها مركبات أخري يمكن أن تكون مفيدة للأنسان في مواجة الملوثات البيئية الأخري المحيطة بنا.