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

Molokhia (Corchorus 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 aminotransferase (ALT), aspartate aminotransferase (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 compounds possessing the antagonistic (phenolic compounds, vitamin A, vitamin C and chlorophyll).

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

INTRODUCTION

Malathion (o.o-dimethyl s-1,2 bis-ethoxy carbonyl ethyl phosphodiithioate), 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 alternation 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 potential of natural plant products as chemopreventive agents present in food consumed by human population (Wanger, 1990; Keiloff 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-soluble 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-(3-malonylglucoside), 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 rats.

**MATERIALS AND METHODS**

**Chemicals**

All chemicals used in this study were obtained from Sigma Chemical Company (St. Louis, USA). Commercial kits were purchased from BioMerieux Company (L’Etoile/France) and from Eagle Diagnostics (Dallas, 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 olitorius L.) leaves were purchased from the local market, pulverized and lyophilized using freeze 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.

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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 Wettstein (1957).

Experimental design

Molathion containing diet was prepared and standardized as described by Banerjee and Hussain (1986). In brief, 20 mg molathion was mixed in 30 ml groundnut oil to produce feed containing 20 ppm molathion. This was incorporated in 1 kg diet and thoroughly mixed to ensure even distribution. Then 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 100 gm/kg bw molokhia, Group III-molathion: rats received 20 ppm molathion along with normal diet and Group IV-molathion + molokhia: rats received 20 ppm molathion along with 100 gm/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 molathion and molokhia on liver was assayed by assayed serum transaminases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities 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 (1970) and serum glutathione-S-transferase (GST) was measured by the method of Habig et al., (1974), using commercial kits purchased from Eagle Diagnostics (Dallas, 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 systems (Kelm, 1993). Free radicals have become an
attractive means to explain the toxicity of numerous xenobiotics. Standard hemato logical 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 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 melokhia supplemented diet as compared to control or malathion-treated group. The intakes of melokhia significantly alleviate the elevation of enzymes activities (P<0.05) in malathion-treated rats. The increased level of ALT, AST and ALP activities 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 melokhia alone or melokhia 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 for 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 melokhia. 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 melokhia alleviated malathion-induced depletion of GSH content and consequently reduced damage in liver.
Fig. 1. Effect of Moikhia 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.
Dietary supplementation 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 (Uroo 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 DOT (Catalrese, 1980). Three cardenolides were isolated from molokhia and showed cytotoxic activities against six cancer lines (Abdel Wahab et al., 1999).

Table 1. Chemical composition of Molokhia.

<table>
<thead>
<tr>
<th>Chemical composition</th>
<th>Fresh</th>
<th>Freeze dried</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture%</td>
<td>80.15±0.00</td>
<td>5.21±0.26</td>
</tr>
<tr>
<td>Protein%</td>
<td>6.63±0.39</td>
<td>30.70±2.71</td>
</tr>
<tr>
<td>Fat%</td>
<td>0.30±0.01</td>
<td>1.94±0.96</td>
</tr>
<tr>
<td>Fiber%</td>
<td>7.50 ± 0.42</td>
<td>33.52±2.52</td>
</tr>
<tr>
<td>Ash%</td>
<td>3.18 ± 0.16</td>
<td>13.90±0.89</td>
</tr>
<tr>
<td>Carbohydrate%</td>
<td>2.39±0.14</td>
<td>15.29±0.78</td>
</tr>
<tr>
<td>Vitamin A (IU)</td>
<td>9500±1357</td>
<td>9500±1350</td>
</tr>
<tr>
<td>Vitamin C (mg/100g)</td>
<td>60.42±4.0</td>
<td>58.66±3.50</td>
</tr>
<tr>
<td>Total soluble phenolic compounds%</td>
<td>4.11±0.2</td>
<td>4.0±0.27</td>
</tr>
<tr>
<td>Total chlorophyll mg/100g</td>
<td>488.57±21.68</td>
<td>487.16±22.51</td>
</tr>
</tbody>
</table>

The other mechanism by which molokhia suppressed the toxicity of malathion may be attributed to the interaction of chlorophyll 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 inhibitors(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


دور الملوخية الوقائي للكلد ضد السمية الناتجة عن مبيد الماثيون

ة زهر

قسم الاقتصاد المنزلي كلية التربية النوعية - جامعة المنوفية

مثير للاهتمام من أثير المبيدات السامة السامة شائعة الاستخدام في جميع أنحاء العالم نهيف حمية

النتائج الإبداعية، وبدأت الزينة والبيئة المحيطية، كلها تجعل في الجسم المثالي والانحلال الوقائي من

الأفريقي بالإضافة إلى استعمال قلق البايرون والقليل، وذلك بعد أن الأسماك لوحده لم يعد

متوفرة نتيجة تناوبها في الأماكن، ولكن، والمدينات، مثل المبيدات، له شار

ملكة على الصحة العامة، ولكن، خطرها في تاثر الصحة وعلى الأجهزة العصبية المركزي والهضمان

( free radicals )

والذي

يدعى بتسمية الملاكية من مواد الكيميائية التي تنتج تأثيرات حرة.

تعرض الأجهزة المتميزة على الكريتونات غير مفيدة في مدارها الخارجي مما يكلفها شرعية

تئناء والثأر، وته هنا نجاح الاحتياجات لسبب الأليافات من الأسماك، والذاتي، يعود

دروعه لتسامح الشوكة.

ذك كان من الأشخاص الناجح مع مركب ثبت نسبة الإصابة في هذه الملاكية، وقد لجأت الأطعماً في início الأحمرية إلى بعض البنادق التي تنبأ الأشخاص في

مالح، وتعزز معها، ونهج للمرافقة لها القرة على حجز ذلك الحرة التي تنتج من السمنات والديالايم

الحمد من تأثيرها على صحة الأشخاص.

فكا كان الدعوة الإبداعية، وذلك، هو نتاج التأثير الوظيفي الشامل في مضادات الأکسدة

الموجودة في تماشى المليئة عند التأثيرات الصارمة لملامس الماثيون على النبات.

هذا، قد تضمة الاضطرابات بتجربة مراقبة على استخدام أدمية مظهر على عدد أربعة أحماض، بما في ذلك نتائجها في أربعة

مجمعة المجموعة الأولى غضب ( التكزيل ) والجامعة الثانية تم تطبيقه على

الملاكية، وذلك بهدف مجاورة لمئة لكل كجم من وزن الراتب والمجمعة الثالثة تحديداً قيمة لكل كجم من الملاكية ومجمعة الثانية، وتم مساحة

الرئية للملامس، ونسبة 20 مليم من الميلKO لكل كجم من الملاكية ورئية المرحلة الناجحة، ثم

متعلقة الجس في ملد المجمعة المليئة تذكرها في المجموعة الثالثة والثانية 100 مليم من الملاكية

الملامس لكل كجم من وزن الراتب إلى الجافة.

وقد أوصت تائج الدراسة أن الماثيون تأثير سام على الكلد حيث أدى إلى زيادة ملائيبعثة، وبذلك، AST (P<0.05) وALT (P<0.05) في مستوي الآلاف الكليسترول ( Al) ومستقبلات الكريتونات ( LP) في مستوي الأكاسين موسم نادر (LP) في مستوي الأكاسين موسم نادر (LP) في مستوي الأكاسين موسم نادر (LP) في مستوي الأكاسين موسم نادر (LP) في مستوي الأكاسين موسم نادر (LP) في مستوي الأكاسين موسم نادر (LP) في مستوي الأكاسين موسم نادر (LP) في مستوي الأكاسين موسم نادر (LP) في مستوي الأكاسين موسم نادر (LP) في مستوي الأكاسين موسم نادر (LP) في مستوي الأكاسين موسم نادر (LP) في مستوي الأكاسين موسم نادر (LP)

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والتالي المقاس المليئة يدل على مراقبة الكلد للملامس، ونسبة 20 مليم من الميلKO لكل كجم من الملاكية ورئية المرحلة الناجحة، ثم

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