EFFECT OF ABAMECTIN ON LIVER FUNCTION AND LIPID PEROXIDATION

Hanan A. Soliman1*, Mohamed B. Ahmed1, Afaf A. El-Kashorey2 and El Moatasem Bellah M. Moawad1

1Department of Chemistry (Biochemistry branch), Faculty of Science, Beni Sueif University, 2Central Agricultural Pesticide Laboratory, Agriculture Research Center, Giza, Egypt

Received: 30/10/2008 Accepted: 30/12/2008

ABSTRACT

The objective of the present study was to evaluate the effect of abamectin on liver function of rats. Abamectin is used as antiparasitic drug in agricultural and domestic animals. Sixty adult male albino rats (150±10g) were classified into 3 groups: Group (1), Control group given tap water; Group (II) Sub-acute group orally treated with \( \frac{2}{3} \) LD\(_{50}\) of abamectin (9.83 mg/kg b.w) for 21 days (4 doses/week) and Group (III) Sub-chronic group orally treated with \( \frac{2}{5} \) LD\(_{50}\) of abamectin (5.93 mg/kg b.w) for 57 days (4 doses/week). Serum aspartate amino transferase (AST), alanine amino transferase (ALT), gamma-glutamyl transferase (GGT) and acid phosphatase as well as hepatic malondiadehyde (MDA), glutathione (GSH), glutathione-S-transferase (GST) and uridine diphosphate glucuronyl transferase (UDPGT) were measured. The results revealed significant increase in serum AST, ALT, ALP and GGT activities in groups II and III, besides increases in alkaline phosphatase in group II compared to the control. The results also showed significant increase in the hepatic MDA in groups II and III compared with the control value. Abamectin also induced a significant inhibition of hepatic GST in groups II and III together with a significant increase of UDPGT activity only in group II compared with the control group. In addition, a non significant change between group II and III was observed with respect to UDPGT activity. In conclusion, the present study indicated that abamectin pesticide may have harmful effects on liver, thus precautions are recommended to avoid its effect on human.

Keywords: Abamectin, liver, lipid peroxidation

*Corresponding author: Hanan A. Soliman, e-mail: hanan_abdelhameid@yahoo.com
INTRODUCTION

Abamectin, a mixture of 22, 23-dihydroavermectin B1a (>or = 80%) and B1b (<or = 20%), is produced by an actinomycete, Streptomyces avermectilis. It is a macrocyclic lactone disaccharide, a member of the avermectins family which is widely used as an antiparasitic drug in agricultural and domestic animals. The compound acts as an insecticide by interfering with the nervous system of the insects, thus they become paralyzed. Avermectin is used to control insect and mite pests of ornamental plants in greenhouses. It is also used in veterinarian medicine for treatment of internal and external parasites and mites of pets and livestock, including scabies. It is also formulated into commercial baits for control of ants and cockroaches (Campbell, 1989).

Abamectins exert their antiparasitic activity via activation of a glutamate-gated chloride channel present in the invertebrate nervous system (Cully et al., 1994). Abamectin is a highly effective and generally well tolerated microfilaricide that may soon become an essential component of many public health initiatives to interrupt transmission of lymphatic filarial infection in an effort to eliminate LF globally (Brown et al., 2000). Previous study has shown that abamectin causes elevation of serum aspartate aminotransferase (AST) (Lowenstein et al., 1996). Zeng et al. (2003) reported that cytochrome p4501 A4 is the major enzyme responsible for avermaectin metabolism in human liver microsomes. Elevation of AST, a cytosolic enzyme of the hepatocytes, reflects the increase of plasma membrane permeability resulting from the damage of hepatocytes and is used to detect liver damage (Klaassen and Baton, 1991). The aim of the present study was to evaluate the effect of abamectin on liver function of rats.

MATERIALS AND METHODS

Animals

Sixty male albino rats, weighing 150±10g raised in the farm of the General Organization of Serum and Vaccnine (Helwan farm) before experimentation. Rats were kept under observation for 2 weeks to exclude any inter-current infection. Rats were housed in plastic cages with metal cover under normal temperature (25±5°C) with 12 h light -
dark cycle (light on at 6.00-18.00 h). They had free access to standard diet and tap water *ad libitum* through out the experiment.

**The pesticide**

The tested pesticide was abamectin (Stromectol, Mectizan), trade name: Vertimec, 1.8 EC, Syngenta Company; used as an acarcide. The product contains avermectin B$_{1a}$ mixture with avermectin B$_{1b}$. Abamectin was emulsified in water immediately before use and orally administered to rats of the experimental groups by esophageal canella.

**Animals grouping**

The rats were divided into three groups (20 rats each). Control group (Group I) included rats orally treated with tap water. Sub-acute group (Group II) in which rats were given orally abamectin at a dose of 9.83 mg/kg b.w ($\frac{2}{3}$ LD$_{50}$) over a period of 21 days (4 doses/week), which represents the highest dose. Sub-chronic group (Group III): pesticide treatment group, animals in this group were given 32 doses of abamectin at 5.93 mg/kg b.w ($\frac{2}{5}$ LD$_{50}$) over a period of 57 days (4 doses/week), which represents the lowest dose. LD$_{50}$ was calculated according the value determined by Hsu *et al.* (2001).

**Blood and tissue sampling**

Blood samples were collected after the end of each experimental period (21 and 57 days) into clean, dry and labeled tubes after decapitation. The tubes contained an anticoagulant heparin (0.1 ml of 5% solutions/5 ml blood) (Schalm, 1986). Blood samples were centrifuged at 3500 rpm for 15 minutes in a refrigerated centrifuge to separate plasma. Samples were kept in a deep freezer at -40°C, till the biochemical measurements were carried out. Samples of liver tissues were taken after scarification of rats by the end of each experimental period. The tissues were rapidly immersed in 10% formalin saline and processed to paraffin sections stained with haematoxylin and eosin for histopathological examination (Lillie, 1965). Another part of the livers were homogenized for certain biochemical investigations.

**Biochemical investigations**

Serum AST and ALT (Reitman and Frankel, 1957) and serum GGT (kinetic method recommended by the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology, 1974) were measured by using commercial kits from Bohringer Manheim Company. Serum ALP (Belfield and Goldberg,
1971) and plasma acid phosphatase (Kind and King, 1954) were determined colorimetrically by using kits purchased from Biomerieux, France. MDA (Uchiyama and Mihara, 1978), the hepatic content of reduced GSH (Mitchell et al., 1973), GST (Chiu and Yu, 1989) and UDPGT (Burchell and Weatherill, 1998) were measured in hepatic tissues by using commercial kits.

RESULTS

As indicated in table (1), serum AST, ALT, ALP and GGT activities were found to be significantly increased in rats treated with abamectin pesticide (group II and III) at the end of both experimental periods (21 and 57 days). The activities of acid phosphatase were statistically increased in group II of animals treated with high dose of Abamectin (21 days) when compared with the control value.

Table (2) indicated the presence of a statistically significant increase (P<0.001) of MDA at both doses of abamectin after 21 and 57 days (group II and III). A significant decrease in GSH content after abamectin administration at high and low doses (group II and III) was also observed compared with those of the control group. Abamectin induced a significant inhibition of GST activity in group II and III compared with the control value. However, abamectin caused a significant increase of UDPGT activity in group II only compared with the control group. A non-significant change between group II and III was observed with respect to UDPGT activity.

Table (1): Effects of oral administration of abamectin pesticide on serum AST, ALT, ALP, GGT and acid phosphatase activities in all studied groups (mean±SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group(I)</th>
<th>Group(II)</th>
<th>Group(III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>82.5±17.8</td>
<td>183.7±14.1*</td>
<td>153±18.7*</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>56.3±12.1</td>
<td>83.5±14.1*</td>
<td>61.9±14.1*</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>93.2±13.8</td>
<td>258.7±41.8*</td>
<td>180.3±36.0*</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>13.7±5.6</td>
<td>26.7±11.0*</td>
<td>22.6±9.9*</td>
</tr>
<tr>
<td>Acid phosphatase (U/L)</td>
<td>10.5±6.6</td>
<td>23.6±8.9*</td>
<td>13.1±7.5*</td>
</tr>
</tbody>
</table>

*P<0.05 (Significant difference with respect to control group)

a P<0.05 (Significant difference with respect to group II)
Table (2): Effects of oral administration of abamectin pesticide on hepatic MDA, GSH, GST and UDPGT levels in all studied groups (mean±SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group (I)</th>
<th>Group (II)</th>
<th>Group (III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol mg protein)</td>
<td>3.4±0.7</td>
<td>4.4±0.9*</td>
<td>3.7±0.4*a</td>
</tr>
<tr>
<td>GSH (mg/gm tissue)</td>
<td>1.1±0.4</td>
<td>0.6±0.1*</td>
<td>0.7±0.2*a</td>
</tr>
<tr>
<td>GST (nmol min/mg (protein)</td>
<td>53.1±10.6</td>
<td>36.6±8.9*</td>
<td>41.9±4.2*a</td>
</tr>
<tr>
<td>UDPGT (nmol min mg protein)</td>
<td>0.5±0.1</td>
<td>0.8±0.2*</td>
<td>0.6±0.1q</td>
</tr>
</tbody>
</table>

*P<0.05 (Significant difference with respect to control group)

P<0.05 (Significant difference with respect to group II)

**DISCUSSION**

The damage of hepatocytes is associated with the alterations of their organelles and morphological change result in changes of various biochemical functions of the liver. This indicates that mechanisms for maintaining the hepatocyte function are very complex. ALP and GGT are the markers normally used to identify cholestasis. The term "serum ALP" is applied to a group of enzymes that catalyze hydrolysis of phosphate esters at an alkaline pH. The enzymes may originate from bone, liver, intestine, kidney or placenta. In children and adolescents, in whom bone growth is active, serum ALP may increase by up to threefold. In patients with hepatobiliary disorders, increased ALP results from increased hepatic production with leakage into the serum rather than from failure to clear or excrete circulating ALP (Keeffe, 1994).

It is considered that abamectin may have a harmful effect on the hepatic cells (Emam and Abd Alla, 2000). Others found that liver contained high residues of abamectin (Roudaut, 1998). In the present study, the effect of abamectin treatment on liver function was monitored by a significant increase in the activities of the transaminase enzymes (ALT and AST), ALP and GGT after 21 and 57 days of treatment. These results were consistent with those reported by Ali et al. (1988) and Ghoneim et al. (1992). However, the results were disagreed with Ewies et al. (1995) and Abd El-Wahab et al. (2002) who found that abamectin caused a decrease in ALT and AST activities in rats. In addition, Gomes et al. (1999) revealed marked decrease in ALT and AST activities as a result of treatment with a mixture of organophosphorous pesticides. The increase of plasma ALT and AST activities in treated animals may be due to the decreased rate
of catabolism of these enzymes in the plasma (Kramer, 1989). Several studies reported that the activities of these enzymes were significantly increased in experimental rats when fed with organophosphorus pesticide and Endrin (Patil et al., 2003; Kalender et al., 2005).

The increase of plasma acid phosphatase recorded in rats treated with abamectin may be associated either with the decrease in stability of liver lysosomal membranes or with tissue damage. Acid phosphatase is associated with lysosomal activity and its elevation reflects proliferation of lysosomes in attempt to sequester the toxic xenobiotic (Ram and Singh, 1988).

The present results also demonstrated that oral treatment of abamectin depleted GSH content and inhibited GST activity. Numan et al. (1990) reported dose and time-dependent depletion of hepatic GSH and glutathione peroxidase by oral treatment of endrin. Similar inhibitory effects on GSH, GST, glutathione peroxidase and glutathione reductase activities in the rat liver due to daily dosing of 15 different classes of pesticides mixture were also reported by Lodovici et al. (1994). The depletion of hepatic GSH level may be due to direct conjugation of GSH with the pesticide and/or their metabolite(s) to detoxify them.

As GST activity is dependent on the availability of GSH as a co-factor, a depleted GSH level may be responsible for subsequent reduction in GSH below a critical concentration, which is known to enhance lipid peroxidation by endogenous promoters, resulting in cell damage (Chaturvedi, 1993). Moreover, there is an increasing evidence of an inverse correlation between susceptibility to chemical carcinogenesis and GST activity and its protective role against lipid peroxidation due to environmental xenobiotics. In the present study, the significant depletion of GSH and inhibition of GST due to Abamectincan also enhance their own toxicity, possibly by diminishing their detoxification. Sultatos et al. (1982) reported increase in the toxicity of parathion and chloropyriphos due to pretreatment of rats and mice with known GSH deviators. It may be possible that GSH depletion in the present study is the result of reduced GSH synthesis, conversion of GSH into GSSG by glutathione peroxidase or, most likely binding of GSH with abamectin and/or their metabolite(s) to render them non-toxic, or interplay of all these suggestions. A strong positive correlation between GSH depletion and
GST inhibition caused by abamectin in the present investigation may be additional support to the hypothesis that GSH depletion by the abamectin insecticides might be the result of their complex formation with GSH as a mode of detoxification.

On the other hand, Siddiqui and Walker (1996) revealed that the same organophosphorous insecticides are metabolized to acids by hepatic microsomal and cytosolic carboxyl esterase in rat. Therefore, an increase in UDPGT activity due to the administration of abamectin to rats could be attributed to detoxify the acid metabolite of the insecticides by conjugation with UDP-glucuronic acid. Ishizuka et al. (1998) reported an increase in UDPGT activity following sub-acute exposure of rats and mice to organophosphorus insecticides, parathion malathion and phosalone. Many investigators observed also a dose-dependent increase in hepatic UDPGT activity in rats and mice due to treatment of various classes of pesticides (Hanioka et al., 1995).

It could be concluded that abamectin pesticide may have harmful effects on liver, thus precautions are recommended during the use of such pesticide to avoid its effect on human. Further researches are also required to investigate the above mentioned hypothesis.

REFERENCES


Effect of Abamectin on Liver Function and Lipid Peroxidation


Effect of Abamectin on Liver Function and Lipid Peroxidation


التأثيرات البيوكيميائية للأفمرميكين على الفئران
حنان عبد الحميد عبد الحفيظ، محمد بسطاوي أحمد، عفاف عبد الحميد الكاشوري، المعتصم بالله محمد مموض

أشعة الكيمياء الحيوية، كلية العلوم، جامعة بني سويف، المركز البحثي الزراعية، الجيزة

الأفمرميكين خليط من 22، 23 ثنائي هيدروافرميكينات أ، ب وينتج من الأكتينومسيتس (ستريبتوميسين) ويستخدم كمبيد حشري في الزراعة وأيضا لكافحة الطفيلات في الحيوانات حيث أنه يؤثر على الجهاز العصبي للحشرة. ويهدف هذا البحث إلى دراسة تأثير الأفمرميكين على وظائف الكبد وبعض الوظائف الأخرى. وقد أجريت هذه الدراسة على ستين من الجرذان البيضاء البالغة (وزنها حوالي 150 جرام) قسمت إلى ثلاث مجموعات (20 جرذ لكل مجموعة) شملت المجموعة الأولى (الضابطة) جرذان تم إعطائها ماء، أما المجموعة الثانية فقد عولجت بالمبيد الحشري (8.3 جرام / كيلو جرام ووزن) لمدة 21 يوم بمعدل 4 جرعات في الأسبوع والمجموعة الثالثة عولمت بالمبيد الحشرى (9.3 جم / كيلو جرام ووزن) لمدة 57 يوما بمعدل 4 جرعات في الأسبوع.

تم أخذ عينات دم من الحيوانات في نهاية التجربة وذلك لقياس إزيمات الكبد (إنزيمات الأدينين والأسيترات أمينوتيروسين-فيزيز) والألفاكيلين ترايس فيزيز والجاما جلوتاميل ترايس بيبيدياز والفيوسفانتاز الحمضية، كما تم تعيين الجلوتاثيول، والجلوتاثيون إس ترايس فيزيز، والإيالونالديميد، والبيرونيل داي فوسفوجولوكو بورونيل ترايس فيزيز في أنسيما الكبد. وقد دلت النتائج على ارتفاع دو دالة إحصائية عالية في نشاط إنزيمات الأدينين والأسيترات أمينوتيروسين-فيزيز والألفاكيلين ترايس فيزيز والجاما جلوتاميل ترايس بيبيدياز في المجموعة الثانية والثالثة بالمقارنة بقيم المجموعة الأولى الضابطة. كما كانت هناك زيادة بشكل إحصائي في مستوى المالونالديميد وتنافس في الجلوتاثيون في المجموعة الأولى والثالثة بالمقارنة بنتائج المجموعة الأولى. كما أظهرت النتائج زيادة في نشاط الجلوتاثيون إس ترايس فيزيز في المجموعة الثانية والثالثة بالمقارنة بفتران المجموعة الأولى مما سبب زيادة هامة في نشاط البيرونيل داي فوسفوجولوكو بورونيل ترايس فيزيز في المجموعة الثانية فقط بالمقارنة بالمجموعة الضابطة.

وقد خلصت هذه الدراسة إلى استنتاج أن الأفمرميكين مبيد حشري يمتلك آثار ضارة على الكبد لذلك يجب اتخاذ الاحتياطات اللازمة لعدم تلوث الإنسان عند استخدام هذا المبيد.