Biochemical alterations in insecticides-treated male albino rats: potential modulatory effects of a standardized aged garlic extract

Nadia M El-Beih1, Gamal Ramadan1*, Mona A Khorsheed2 & Rehab SA Ahmed2

1Zoology Department, Faculty of Science, Ain Shams University, Abbasiya Sq., Cairo 11566, Egypt;
2Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Food (QCAP Lab), Giza, Egypt
E-mails: gamal_ramadan@hotmail.com; gramadan@sci.asu.edu.eg

Received 04 August 2016, revised 02 December 2016

Pesticides poisoning is a major clinical problem worldwide. Malathion (an organophosphate insecticide) and carbaryl (a carbamate insecticide) are widely used and pose a potential health hazard for both humans and animals. They are common insecticides residue found in food, especially in developing countries. Here, we investigated some biochemical alterations related to dyslipidemia, tissue injury and the impairment in liver and kidney functions in male albino rats treated with 0.1 LD50 of malathion (89.5 mg/kg body weight) and/or carbaryl (33.9 mg/kg body weight), as well as, evaluated the potential modulatory effects of 200 mg/kg body weight of a standardized odorless (free from allicin) Kyolic aged garlic extract (AGE, containing 0.147 % of its major active constituent S-allylcysteine) on the resulted toxicity. Doses were orally administered to animals for four consecutive weeks. The present study showed that AGE significantly alleviated (P<0.05-0.001) most insecticides toxicity in rats through modulating the body-weight loss and hepatomegaly, blood dyslipidemia and the elevation in atherogenic indices, blood hyperbilirubinemia, hyperglycemia and hypoalbuminemia, the impairment in kidney function (by decreasing renal insecticides residue), and oxidative liver damage (by augmenting hepatic glutathione antioxidant-system). Thus, AGE may be useful as a dietary adjunct in highly vulnerable subjects to insecticides intoxication.

Keywords: Dyslipidemia, Hepatotoxicity, Insecticides, Kidney dysfunction, Kyolic AGE.

IPC Int. Cl.8: A01N, A61K 39/00, A01D 15/00, A01D 20/00

Pesticides poisoning is a major clinical problem worldwide, especially in developing countries1. Subjects living in proximity to farms and those exposed heavily to home application of pesticides or eat food rich in pesticides residue, in addition to workers of pesticide manufactories, agriculture workers and their families are highly vulnerable to pesticides intoxication2. Malathion, the oldest and most extensively used organophosphate insecticides throughout the world, is used to control the pests of forests, agricultural crops, stored grains, ornamental plants, greenhouses and gardens3,4. In addition, malathion is sprayed in large doses to control livestock pests, household insects and human lice4. On the other hand, carbaryl is the universal yard and garden carbamate insecticide that is considered one of the strongest insecticides widely used to control pests in agriculture6. It is a major component of many pesticides formulation and is also formulated as slug and snail baits. Malathion and carbaryl is common in insecticides residue found in food, especially in developing countries. They act as esterases inhibitors, mainly acetylcholinesterase inhibitors, causing severe side effects on central and peripheral nervous system. However, many non-anticholinesterase side effects of malathion and carbaryl were recently reported in animals including oxidative tissue injury as well as endocrine and metabolic alterations3,6-8.

Garlic (Allium sativum L., family Alliaceae) has had a worldwide reputation since ancient time as a valuable prophylactic agent and a popular remedy for several types of morbidities and disturbances in homeostasis8. However, chronic administration of raw garlic may lead to indigestion, peptic ulcers and anemia, and its pungent odor (allicin) lingers on the breath and skin causing a social deterrent10-12. A unique odorless (free from allicin) garlic preparation called aged garlic extract (AGE) has been reported to possess an array of pharmacologic effects, which were attributed to its high content of water-soluble organosulfur compounds, especially S-allylcysteine (SAC, the major active constituent of AGE), without

*Corresponding author
inducing the adverse effects shown with odorous garlic preparation\textsuperscript{9,11-13}. Our previous studies found that 200 mg/kg body weight of AGE was effective against experimentally-induced genotoxicity, immunosuppression and systemic anaphylaxis\textsuperscript{12,14}. In addition, we found that 200 mg/kg body weight of AGE was effective in alleviating the normocytic normochromic anemia and the delay in the skin-burning healing process in insecticide-treated rats\textsuperscript{15}. In this context, we hypothesized that AGE may have protective effects against biochemical alterations in highly vulnerable subjects to insecticides intoxication. Therefore, the objective of this study was to evaluate the modulatory effects of a standardized Kyolic AGE on the potential toxicity induced by malathion and carbaryl (separately or together) in male albino rats, especially with reference to metabolic disorders and the risk for atherosclerosis as well as the impairment in vital organs function (liver and kidney).

**Methodology**

**Insecticides**

Malathion (C\textsubscript{10}H\textsubscript{19}O\textsubscript{6}PS\textsubscript{2}, an emulsifiable concentrate) and carbaryl (C\textsubscript{12}H\textsubscript{11}NO\textsubscript{2}, a wettable powder) were purchased from El-Nasr Co. for Intermediate Chemicals (Giza, Egypt) and May Trade S.A.E. (Giza, Egypt), respectively.

**Aged garlic extract**

A standardized Kyolic AGE (Formula-100) capsules were purchased from Wakunaga of America Co., Ltd. (Mission Viejo, CA, USA). Kyolic AGE is prepared by soaking organic sliced raw garlic cloves in 15-20\% ethanol for 20 months at room temperature. This process converts harsh unstable compounds, such as allicin, to stable health-promoting substances such as SAC and S-allylmercaptocysteine. The extract is then filtered and concentrated under reduced pressure at low temperature. The extract had 305 gm/L of extracted solids and the concentration of SAC (the most abundant water-soluble organosulfur compound in AGE) was 1.47 gm/L\textsuperscript{16}.

**Animals**

Adult male Wistar albino rats (Rattus norvegicus), weighing 125-135 gm, were obtained from the National Research Center (Giza, Egypt). The animals were housed in suitable cages and acclimatized to laboratory conditions for a period of 1 week before the commencement of the experiments. The rats were fed standard rodent food pellets (Agricultural-Industrial Integration Company, Giza, Egypt) and double-distilled water. All animals were humanely treated in accordance with the WHO guidelines for animal care, and the study design was approved by the Ain Shams University Research Ethics Committee. As estimated in the present study, the oral median lethal dose (LD\textsubscript{50}) of malathion and carbaryl in rats was 895 and 339 mg/kg body weight, respectively. Here, we used a dose of 0.1 LD\textsubscript{50} of insecticides to reflect their small amounts found in the contaminated food and to be able to study the synergistic/antagonistic effects of the tested insecticides.

**Experimental design and treatment schedule**

Experimental animals were randomly divided into 8 groups of 5 rats each. Animals were given, orally (by gavage) and daily for 4 weeks, either AGE (200 mg/kg body weight, suspended in 0.5 mL distilled water)\textsuperscript{15} alone or 0.1 LD\textsubscript{50} of insecticide(s), suspended in 0.5 mL distilled water, with/without AGE. The control animals were not given AGE or any insecticide, but received 0.5 mL distilled water (as vehicle) orally (by gavage) and daily for 4 weeks and housed under the same conditions of treated animals.

**Blood and tissues sampling**

Animals were fasted overnight and subjected to light diethyl ether anesthesia before killing on day 29. Blood was collected into clean and dry test-tubes without anticoagulant agent to separate serum, which was divided into samples and preserved at −70 °C until used for biochemical analysis. Immediately after killing the animals, the liver and kidney were separated out of the body, cleaned, weighed, rapped in aluminum foil and kept frozen at −70 °C until used for the determination of tissue insecticides residue by gas chromatography techniques using HP 6890 gas chromatography with an electron capture detector (Hewlett-Packard Company, Wilmington, DE, USA) according to the manufacturer’s recommendations. Another portion of the liver was homogenized in 5 mL cold buffer (0.1 M-phosphate buffer, pH 7.4) per gram tissue. Then, the homogenate was stored at −70 °C until used for the determination of total glutathione and reduced glutathione (GSH) contents according to the method of Saville\textsuperscript{17} and Prins & Loose\textsuperscript{18}, respectively. Liver oxidized glutathione (GSSG) content was calculated as follows: GSSG = Total glutathione − GSH.
Measurements

Body-weight change was calculated by the following equation: body-weight change = body weight at the end of the experiment – body weight at the beginning of the experiment. Serum albumin, bilirubin (total and direct), glucose, triacylglycerol, total cholesterol, high-density lipoprotein (HDL) cholesterol, urea and creatinine concentrations as well as alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) activities were colorimetrically determined by commercial kits (Bio-Diagnostic, Giza, Egypt). Serum indirect bilirubin concentration was calculated as follows: indirect bilirubin = total bilirubin – direct bilirubin. Serum low-density lipoprotein (LDL) cholesterol concentration was calculated according to the equation of Friedewald et al.\textsuperscript{19}.

\[ \text{LDL cholesterol} = \frac{\text{total cholesterol} - (\text{triacylglycerol}/5) - \text{HDL cholesterol}}{\text{Friedewald et al.}} \]

Atherogenic indices were calculated as follows: atherogenic index (1) = total cholesterol: HDL cholesterol ratio, atherogenic index (2) = LDL cholesterol: HDL cholesterol ratio.

Results

AGE alleviated the changes in body weight and organs relative weights, and decreased tissue insecticides residue, in insecticides-treated rats

The present study showed that treatment of rats with AGE alone did not significantly alter \((P > 0.05)\) the body-weight gain and the relative weight of liver and kidney compared with the control animals (Figs. 1a & b). Also, treatment of rats with malathion alone and carbaryl alone did not significantly alter \((P > 0.05)\) the body-weight gain, but malathion plus carbaryl-treated rats showed a severe body-weight loss (172.5 %, \(P < 0.001\)), compared with the control animals (Fig. 1a). On the other hand, treatment of rats with malathion and carbaryl (separately or together) caused a significant increase in the relative weight of liver and kidney (22.1 ± 6.2 %, \(P < 0.05-0.001\)) compared with the control animals (Fig. 1b). Insecticides residue was not detected in liver, but was detected only in renal tissues of all insecticides-treated animals and significantly increased \((P < 0.001)\) in rats treated with carbaryl in presence or absence of malathion compared with the control animals (Fig. 1c). Most of the above adverse effects of insecticides were completely modulated and reverted to near normal values by AGE, except the loss in body weight and the increase in liver relative weight in malathion plus carbaryl-treated rats were partially modulated by the AGE, \(P < 0.01-0.001\) and \(P < 0.001\) compared with the control and insecticides-only-treated animals, respectively (Fig. 1).

Fig. 1—Body-weight change (a), liver and kidney relative weights (b), renal insecticides residue (c) of insecticide(s) with/without aged garlic extract (AGE)-treated rats. Values are means, with their standard errors represented by vertical bars. Insecticides residue was not detected in liver. CAR, carbaryl; MAL, malathion. Mean values were significantly different from that of the control group: \(*P < 0.05\), \(**P < 0.01\), \(***P < 0.001\); mean values were significantly different from that of the group treated with the same insecticide(s) without AGE: \(†P < 0.05\), \(†††P < 0.001\) (One-way ANOVA with Bonferroni’s multiple comparison test, \(n = 5\)).
AGE alleviated the metabolic disorders in insecticides-treated rats

The present study showed that treatment of rats with AGE alone did not significantly alter \((P > 0.05)\) serum albumin and glucose concentrations, lipid profile and atherogenic indices compared with the control animals (Table 1). On the other hand, treatment of rats with malathion and carbaryl (separately or together) significantly increased serum glucose, triacylglycerol, total cholesterol and LDL cholesterol concentrations as well as atherogenic indices (59.2 ± 13.7 %, \(P < 0.01-0.001\)), except that serum total cholesterol and LDL cholesterol concentrations did not significantly change \((P > 0.05)\) in malathion-only-treated animals, compared with the control animals (Table 1). In addition, treatment of rats with malathion and carbaryl (separately or together) significantly decreased serum albumin and glucose, triacylglycerol, total cholesterol and LDL cholesterol concentrations as well as atherogenic indices (59.2 ± 13.7 %, \(P < 0.01-0.001\)). Most metabolic disorders that were induced by treatment of rats with malathion and carbaryl (separately or together) including hypoalbuminemia, hyperglycemia, dyslipidemia and the increase in atherogenic indices were significantly modulated by AGE, \(P < 0.05-0.001\) compared with the insecticide(s)-only-treated animals (Table 1). The modulatory activity of AGE on metabolic disorders induced by insecticides was complete and the values reverted to near normal levels \((P > 0.05)\) in rats treated with malathion alone and carbaryl alone, but was mostly partial \((P < 0.05-0.001)\) in malathion plus carbaryl-treated rats, compared with the control animals (Table 1).

AGE alleviated hepatotoxicity and the impairment in kidney functions in insecticides-treated rats

The present study showed that treatment of rats with AGE alone induced a significant increase in liver total and reduced glutathione concentrations as well as GSH:GSSG ratio (24.7 ± 17.9 %, \(P < 0.001\)) and a significant decrease in liver GSSG concentration (30.1 %, \(P < 0.001\)), but did not significantly alter \((P > 0.05)\) serum bilirubin, urea and creatinine concentrations as well as cellular toxicity markers (serum ALAT, ASAT, ALP and LDH activities), compared with the control animals (Table 2 & Fig. 2). On the other hand, treatment of rats with malathion and carbaryl (separately or together) significantly increased serum total and direct bilirubin, urea and creatinine concentrations as well as cellular toxicity markers (serum ALAT, ASAT, ALP and LDH activities), compared with the control animals (Table 2 & Fig. 2).

### Table 1—Serum albumin and glucose concentrations, lipid profile, and atherogenic indices of insecticide(s) with/without aged garlic extract (AGE)-treated rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>AGE</th>
<th>MAL</th>
<th>CAR</th>
<th>MAL + CAR</th>
<th>AGE +</th>
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</thead>
<tbody>
<tr>
<td>Albumin (gm/L)</td>
<td>35.8 ± 0.9</td>
<td>31.4 ± 0.7</td>
<td>27.4 ± 1.3</td>
<td>30.6 ± 1.8</td>
<td>26.6 ± 0.9</td>
<td>33.4 ± 0.7</td>
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<tr>
<td>Glucose (mg/L)</td>
<td>1429 ± 12.1</td>
<td>1430 ± 11.1</td>
<td>1490 ± 10.4</td>
<td>1490 ± 6.8</td>
<td>1608 ± 12.5</td>
<td>1464 ± 7.6</td>
</tr>
<tr>
<td>Triacylglycerol (mg/L)</td>
<td>397.6 ± 14.2</td>
<td>396.8 ± 13.2</td>
<td>549.6 ± 9.8</td>
<td>540.2 ± 4.7</td>
<td>693.0 ± 7.6</td>
<td>433.0 ± 4.9</td>
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<tr>
<td>Total cholesterol (mg/L)</td>
<td>521.4 ± 10.3</td>
<td>522.0 ± 9.9</td>
<td>526.4 ± 9.6</td>
<td>624.2 ± 9.0</td>
<td>576.8 ± 5.4</td>
<td>523.6 ± 9.1</td>
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<td>HDL cholesterol (mg/L)</td>
<td>293.0 ± 2.5</td>
<td>293.2 ± 1.7</td>
<td>232.2 ± 2.2</td>
<td>229.8 ± 3.7</td>
<td>172.4 ± 3.5</td>
<td>291.6 ± 1.7</td>
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<td>LDL cholesterol (mg/L)</td>
<td>148.9 ± 10.9</td>
<td>149.4 ± 10.8</td>
<td>184.3 ± 9.7</td>
<td>286.4 ± 13.0</td>
<td>265.8 ± 4.6</td>
<td>145.4 ± 7.9</td>
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<tr>
<td>Atherogenic index (1)</td>
<td>1.78 ± 0.04</td>
<td>1.78 ± 0.03</td>
<td>2.27 ± 0.06</td>
<td>2.72 ± 0.08</td>
<td>3.35 ± 0.07</td>
<td>1.80 ± 0.03</td>
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<tr>
<td>Atherogenic index (2)</td>
<td>0.508 ± 0.040</td>
<td>0.508 ± 0.037</td>
<td>0.796 ± 0.045</td>
<td>1.250 ± 0.076</td>
<td>1.542 ± 0.049</td>
<td>0.502 ± 0.027</td>
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</tbody>
</table>

Values are means with their standard errors. CAR, carbaryl; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MAL, malathion. *Total cholesterol:HDL cholesterol ratio, bLDL cholesterol:HDL cholesterol ratio. Mean values were significantly different from that of the control group: *\(P < 0.05\), **\(P < 0.01\), ***\(P < 0.001\); mean values were significantly different from that of the group treated with the same insecticide(s) without AGE: †\(P < 0.05\), ††\(P < 0.01\), †††\(P < 0.001\) (One-way ANOVA with Bonferroni’s multiple comparison test, \(n = 5\)).
Liver total glutathione, reduced glutathione (GSH) and oxidized glutathione (GSSG) concentrations, and GSH:GSSG ratio (a-d, respectively) of insecticide(s) with/without aged garlic extract (AGE)-treated rats. Individual values are shown, with means represented by horizontal bars. CAR, carbaryl; MAL, malathion. Mean values were significantly different from that of the control group: *P < 0.05, **P < 0.01, ***P < 0.001; mean values were significantly different from that of the group treated with the same insecticide(s) without AGE: †P < 0.05, ††P < 0.01, †††P < 0.001 (One-way ANOVA with Bonferroni’s multiple comparison test, n = 5).

Values are means with their standard errors. ALAT, alanine aminotransferase; ASAT, aspartate transaminase; CAR, carbaryl; LDH, lactate dehydrogenase; MAL, malathion. Mean values were significantly different from that of the control group: *P < 0.05, **P < 0.01, ***P < 0.001; mean values were significantly different from that of the group treated with the same insecticide(s) without AGE: †P < 0.05, ††P < 0.01, †††P < 0.001 (One-way ANOVA with Bonferroni’s multiple comparison test, n = 5).

Table 2—Serum bilirubin concentration, cellular toxicity markers and kidney functions of insecticide(s) with/without aged garlic extract (AGE)-treated rats

<table>
<thead>
<tr>
<th>Serum bilirubin (mg/L)</th>
<th>Control</th>
<th>AGE</th>
<th>MAL</th>
<th>CAR</th>
<th>MAL + CAR</th>
<th>AGE + CAR</th>
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<tbody>
<tr>
<td>Total</td>
<td>14.0 ± 0.3</td>
<td>13.9 ± 0.3</td>
<td>15.8 ± 0.3</td>
<td>15.8 ± 0.4</td>
<td>23.6 ± 0.4</td>
<td>14.6 ± 0.2</td>
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<td>Direct</td>
<td>12.6 ± 0.2</td>
<td>12.4 ± 0.2</td>
<td>14.2 ± 0.3</td>
<td>14.3 ± 0.2</td>
<td>22.5 ± 0.2</td>
<td>13.2 ± 0.2</td>
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<tr>
<td>Indirect</td>
<td>1.4 ± 0.3</td>
<td>1.5 ± 0.4</td>
<td>1.6 ± 0.5</td>
<td>1.4 ± 0.4</td>
<td>1.1 ± 0.5</td>
<td>1.4 ± 0.1</td>
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<tr>
<td>Cellular toxicity markers</td>
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<tr>
<td>Serum ALAT activity (IU/L)</td>
<td>47.9 ± 1.3</td>
<td>47.8 ± 0.7</td>
<td>55.4 ± 1.4</td>
<td>53.8 ± 1.7</td>
<td>57.0 ± 1.3</td>
<td>49.6 ± 1.0</td>
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<td>Serum ASAT activity (IU/L)</td>
<td>121.8 ± 3.2</td>
<td>121.9 ± 3.1</td>
<td>142.5 ± 5.6</td>
<td>141.4 ± 5.0</td>
<td>146.5 ± 4.3</td>
<td>123.3 ± 3.4</td>
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<tr>
<td>Serum ALP activity (IU/L)</td>
<td>49.7 ± 0.8</td>
<td>49.5 ± 0.9</td>
<td>55.0 ± 1.6</td>
<td>54.8 ± 1.5</td>
<td>56.4 ± 1.5</td>
<td>51.4 ± 0.5</td>
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<tr>
<td>Serum LDH activity (IU/L)</td>
<td>333.8 ± 2.3</td>
<td>337.6 ± 1.7</td>
<td>352.2 ± 2.5</td>
<td>347.0 ± 2.6</td>
<td>354.6 ± 2.4</td>
<td>340.0 ± 3.1</td>
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<tr>
<td>Kidney functions</td>
<td></td>
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<td>Serum urea (mg/L)</td>
<td>428.0 ± 8.6</td>
<td>408.0 ± 8.4</td>
<td>520.0 ± 7.7</td>
<td>468.0 ± 9.0</td>
<td>552.0 ± 8.3</td>
<td>454.0 ± 10.6</td>
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<tr>
<td>Serum creatinine (mg/L)</td>
<td>3.96 ± 0.05</td>
<td>3.94 ± 0.05</td>
<td>4.36 ± 0.07</td>
<td>4.34 ± 0.09</td>
<td>4.74 ± 0.05</td>
<td>4.26 ± 0.09</td>
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Fig. 2—Liver total glutathione, reduced glutathione (GSH) and oxidized glutathione (GSSG) concentrations, and GSH:GSSG ratio (a-d, respectively) of insecticide(s) with/without aged garlic extract (AGE)-treated rats. Individual values are shown, with means represented by horizontal bars. CAR, carbaryl; MAL, malathion. Mean values were significantly different from that of the control group: *P < 0.05, **P < 0.01, ***P < 0.001; mean values were significantly different from that of the group treated with the same insecticide(s) without AGE: †P < 0.05, ††P < 0.01, †††P < 0.001 (One-way ANOVA with Bonferroni’s multiple comparison test, n = 5).
rats (Table 2 & Fig. 2). Moreover, liver total and reduced glutathione concentrations as well as GSH:GSSG ratio significantly increased (25.4 ± 17.6 %, \( P < 0.001 \)) above the normal values, while liver GSSG concentration significantly decreased (30.0 %, \( P < 0.001 \)) below the normal value, in malathion plus AGE-treated rats (Fig. 2).

**Discussion**

Results obtained here indicated that rats treated with malathion and carbaryl (separately or together) displayed hyperglycemia and dyslipidemia, which could be termed as primary risk factors for initiation and progression of atherosclerotic lesions and subsequent cardiovascular complications. Dyslipidemia also results in liver steatosis and enhances oxidative damage to hepatocyte membrane leading to leakage of endogenous enzymes into circulation. Indeed, in the present study, the insecticides-treated rats showed hepatomegaly as well as elevation in atherogenic indices and markers for cellular toxicity, which resulted (entirely or in part) from dyslipidemia, deficiency in hepatic glutathione antioxidant-system, and oxidative liver injury. Other studies reported that liver macrovacuolar steatosis and oxidative injury were found in insecticides-treated rats. Also, our previous study reported that malathion and/or carbaryl induced prolongation of clotting time, which may be attributed to a reduction in clotting factors production resulting from liver toxicity. On the other hand, the elevation in serum total and direct bilirubin concentrations shown here in insecticides-treated rats indicated a problem associated with the decrease in elimination of bilirubin by the liver cells and/or blockage of the bile ducts as commonly found in hepatitis and bile duct gallstones, respectively.

In the present study, AGE significantly modulated (\( P < 0.05-0.001 \)) hepatomegaly, hyperbilirubinemia, hyperglycemia, dyslipidemia, and the elevation in atherogenic indices and cellular toxicity markers in insecticides-treated rats. The antidiagnostic activity of AGE may explain its alleviative effect on the hepatomegaly and the impairment of liver functions shown in insecticides-treated rats. In addition, the marked decrease (\( P < 0.01-0.001 \)) in atherogenic indices induced by AGE in insecticides-treated rats suggested that AGE may reduce the incidence of atherosclerosis in highly vulnerable subjects to insecticides intoxication. Recently, it was reported that AGE might be a useful natural therapeutic intervention to increase adiponectin, a protective adipocytokines with antidiabetic, anti-inflammatory and antiatherogenic effects, and hence prevent cardiovascular complications in individuals with metabolic syndrome. Also, Amdred reported that both AGE and its SAC were effective in reducing dyslipidemia and scavenging oxidative free radicals induced by high-fat diet in experimental animals, probably through increasing the release of endothelium bound lipoprotein lipase (which hydrolyses the triacylglycerols into free fatty acids) and the activity of lecithin-cholesterol acyltransferase (which contributes in regulation of blood lipids). In addition, Balamash et al. reported that daily dietary intervention with AGE to diabetic patients alleviated the hypertriglyceridemia. Other studies demonstrated that AGE and its SAC showed antihyperglycemic and antidiabetic effects and decreased serum ASAT, ALAT and ALP activities to near normal levels in rats treated with streptozotocin-induced diabetes. AGE scavenged oxidants, inhibited lipid peroxidation, inflammatory prostaglandins and platelet aggregation, reduced hepatic cholesterol synthesis and arterial plaque formation, lowered blood pressure, and increased microcirculation, which were protective from cardiovascular disorders in diabetes. In addition, AGE and its organosulfur compounds showed protective ability against lipid peroxidation, liver toxicity and the risks for cardiovascular disorders caused by a variety of medicinal and environmental substances. Recently, Shin et al. reported the hepatoprotective effects of AGE in rodent models of liver injury caused by either carbon tetrachloride or D-galactosamine, and suggested that AGE supplementation might be a good adjuvant therapy for the management of hepatotoxicity. All of these reports explained the hepatoprotective and cardioprotective effects of AGE shown in the present study in insecticides-treated rats.

Depletion of hepatocytes GSH (the first line defense against lipid peroxidation) in insecticides-treated rats probably resulted from the excessive generation of reactive oxygen species in the form of hydrogen peroxide through the oxidation of insecticides by cytochromes P450 and flavin-containing monooxygenases in order to facilitate their excretion from the body. In addition, depletion of hepatocytes GSH rendered them more susceptible to
tumor necrosis factor-α induced apoptosis, which may explain the elevation in serum transaminases activity in insecticides-treated rats. Cysteine is an important amino acid for GSH formation, and AGE is rich in cysteine-containing compounds such as SAC and S-allylmercaptocysteine. This may explain the obtained beneficial effect of AGE on hepatic glutathione antioxidant-system of insecticides-treated rats. Also, El-Beih et al. found that AGE significantly improved hepatic glutathione antioxidant-system in acetaldehyde-intoxicated rats. The severe decrease in the body weight shown in the present study in rats treated with malathion and carbaryl probably resulted from a decrease in food intake (data not shown) and the degeneration of muscle tissues to compensate for the energy lost from the body due frequent urination in uncontrolled hyperglycemia. Also, Al-Gehani found a decrease in the body weight and food intake in insecticide-treated quail. On the other hand, our data showed that AGE partially, but significantly (P < 0.001), modulated the body-weight loss in insecticides-treated rats, which may have resulted from improving food intake and glucose metabolism. Also, Uda et al. reported that AGE modulated the decrease in the body weight in rats treated with diethyl nitrosamine-induced liver carcinoma.

Kidney injury and the elevation in serum urea and creatinine concentrations have been noticed in rats treated with organophosphate and carbamate insecticides in the present study and other studies. The renal toxicity that was induced by insecticides, such as carbamate, in both humans and experimental animals begins with changes in glomerular function, followed by acute morphologic changes that may slowly progress to a chronic irreversible nephropathy. The inhibitory effects of AGE on the increase in kidney relative weight of insecticides-treated rats may be due to improvement of kidney functions resulting from its ability to decrease the amount of insecticides residue and free radicals in renal tissues. It was also reported that AGE and its SAC significantly protected rats from cyclosporine-induced nephrotoxicity and reduced serum urea and creatinine concentrations through inhibiting the generation of nitrogen reactive species such as nitric oxide. In addition, other studies reported the renoprotective effect of AGE and SAC in streptozotocin-induced diabetes and 5/6 nephrectomized rats, respectively. In the present study, the modulatory effects of AGE on serum hypoalbuminemia of insecticides-treated rats may have resulted from its ability to increase the synthesis of albumin and/or decrease the excretion of albumin by improving liver and kidney functions, respectively. Also, El-Beih et al. found that AGE significantly modulated the decrease in serum albumin concentration in rats treated with acetaldehyde-induced liver toxicity.

In conclusion, the efficacy of carbaryl in inducing dyslipidemia was slightly more than that of malathion, which had a slightly more efficacy in decreasing serum albumin concentration and elevating serum urea and creatinine concentrations as well as ALAT and LDH activities. In addition, the use of both insecticides showed a synergistic effect. On the other hand, AGE was effective in alleviating most adverse effects induced in rats by malathion and carbaryl and thus it may be useful as a dietary adjunct for alleviating the toxicity in highly vulnerable subjects to insecticides intoxication.

Acknowledgment
This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. The authors have no potential financial conflict of interest. N M E-B and G R planned the study, designed all experiments, and summarized, discussed and interpreted the results. R S A A carried out all experiments (except the determination of tissue insecticides residue by gas chromatography, which was carried out by M A K) and performed the statistical analysis with assistance from G R who drafted the manuscript.

References


17 Saville B, A scheme for the colorimetric determination of microgram amounts of thiols, Analyst, 83 (1958) 670-672.


