Hepatotoxicity and Nephrotoxicity in Mice Induced by Abamectin and Ameliorating Effect of Quercetin

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ABSTRACT---- Abamectin is a natural fermentation product and widely used as insecticide, acaricide and anthelmintic drug. This study was conducted to investigate the acute toxicity of abamectin toward male mice and evaluate the impact effect of repeated sublethal dose (1/10 LD₅₀ for 14 days) on some hematological, immunological and biochemical parameters as well as histopathological changes. Also, the effectiveness of quercetin in alleviating the toxicity of abamectin was investigated. The data indicated that, there was significant decrease in the body weight gain and increase in the relative weights of liver and kidney of animals treated with abamectin compared with control. Also, a significant decline in hemoglobin content (Hb), packed cell volume (PCV) and red blood cells (RBC), while the white blood cells (WBC), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) increased. Abamectin reduced the cellular immunity; active and total rosette-forming cells (RFC) and humoral immunity; plaque forming cells (PFC) and serum immunoglobulins; total Ig, IgG and IgM levels. Oxidative stress of abamectin was assessed by significant increase of lipid peroxidation (LPO), glutathione peroxidase (GPx) activity and decrease of glutathione content (GSH). Also, the function parameters of liver; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activities and kidney; creatinine and urea concentrations were significantly increased by abamectin treatment. The above findings were confirmed by histopathological examination of liver and kidney. In addition, the results showed that quercetin supplementation significantly protected the adverse effects of abamectin.

Keywords--- Abamectin; quercetin; oxidative stress; histopathology; immunology; biochemical parameters

1. INTRODUCTION
Abamectin is obtained by natural fermentation of Streptomyces avermitilis, which provides a mixture of avermectins consisting of 80 % of avermectin B1a and 20 % of avermectin B1b. Abamectin is currently used as a pest control agent in livestock and as an active principle of nematicides and insecticides for agricultural use.[¹] Abamectin has been used extensively all over the world and is still one of the most commonly used pesticides in Egypt. A biomarker may be any measurable biochemical, cellular, physiological or behavioral change in an organism or population that indicates exposure to chemical pollutants.[²] By focusing on the intermediate, sublethal effects of a pollutant, this developing field aims to reveal environmental threats before obvious toxic effects such as death of organisms are observed. Changes in body weight have been used as an indicator of adverse effects of drugs and
chemicals. Also, organ weight changes have long been accepted as a sensitive indicator of chemically induced changes to organs and in toxicological experiments.[3] Hematological parameters in general are commonly used in disease diagnosis in mammals health practice[4], they usually reflect the physiological responsiveness of the animal to its external and internal environments and this serves as a veritable tool for monitoring animal health.[5] The immune system is important for defense against a variety of pollutants. The early work in this area was reviewed by Ercegovich.[6], since that time there has been a growing interest in evaluating pesticides effects on a number of different aspects of the immune response.[7]

The toxic manifestations induced by pesticides may be associated with the enhanced production of reactive oxygen species (ROS), which give an explanation for the multiple types of toxic responses.[8] The production of ROS is to be caused by a mechanism in which xenobiotics, toxicants and pathological conditions may produce oxidative stress and induce various tissue damage i.e., liver, kidney and brain.[9] The cells have different mechanisms to alleviate oxidative stress and repair damaged macromolecules. The primary defense is offered by enzymatic and non-enzymatic antioxidants, which have been shown to scavenge free radicals. Antioxidants, can prevent the uncontrolled formation of free radicals or inhibit their reaction with biological sites, also the destruction of most free radicals rely on the oxidation of endogenous antioxidants mainly by scavenging and reducing molecules.[8]

In toxicity studies, a variety of biochemical parameters are measured to evaluate a broad range of physiological and metabolic functions affecting target organ identification and tissue injury assessment.[10] A combination of some common biochemical parameters provide better information from pattern recognition, e.g. enzymes like ALT and AST for hepatotoxicity, and urea and creatinine for glomerular function.[11]

Quercetin one of the most abundant natural flavonoids, is present in large amounts in vegetables, fruits, tea, and olive oil.[12] Quercetin can act by scavenging free radicals, chelation of divalent cations, inhibition of some enzymes and protecting against DNA damage.[13] Because of the health problems induced by many environmental pollutants, much effort has been expended in evaluating the relative antioxidant potency of quercetin. Therefore, the present study planned to investigate the effect of consecutive repeated sublethal dose of abamectin for 14 day on some hematological, immunological and biochemical parameters besides the histological changes of male mice. In addition, the role of quercetin in overcoming the damage induced by abamectin in male mice was investigated.

2. MATERIALS AND METHODS

Chemicals:
Abamectin technical grade (96.4 %) was kindly supplied by the group of Hailir Pesticides and Chemicals Co. Quercetin, 98 % and all other chemicals used in this study were of the highest purified grades purchased from Simga-Aldrich and Marck Chemical Companies.

Animals:
Male Swiss albino mice (Mus musculus) each weighting, 20 ± 5 gm were obtained from Faculty of Science, Alexandria University, Alexandria, Egypt. Animals were housed in stainless still cages under the laboratory conditions of 25 ± 5 ºC and 70 ± 10 % humidity with 12 h dark: light cycle, provided with diet and water ad libitum. The animals were allowed to acclimatize for two week prior to the start of the study. All the experiments were carried out in compliance with the guide for the care and use of laboratory animals. The experimental protocols were approved by the Ethical Committee for the use of laboratory animals.
Determination of the LD50:
Different concentrations of abamectin were dissolved in corn oil and administered to mice orally with a single dose via gavage. Mice serving as control had received corn oil only. The acute toxicity of abamectin was calculated according to Weill [14] and was expressed as LD50 value and its confidence limits.

Experimental design:
The mice were divided into four groups, each including five animals and daily treated orally for 14 consecutive days as follows:
Group 1: Corn oil used as control.
Group 2: Quercetin alone (50 mg/kg bw).
Group 3: Abamectin alone in a dose equivalent to 1/10 LD50.
Group 4: Abamectin (1/10 LD50) plus quercetin (50 mg/kg bw).

At the end of experiment, blood was collected into two tubes. The first aliquot drawn in lithium-heparinized tube, another aliquot was taken in plain tube for serum preparation. Then liver, kidney and spleen were quickly removed and weighed individually.

Hematological studies:
Blood samples were analyzed for hemoglobin content (Hb), % packed cell volume (PCV), red blood cells (RBC's) and white blood cells (WBC's) count as described by Dacie and Lewis,[15] Erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated.

Immunological studies:
All mice were immunized by a single subcutaneous injection (20 % sheep red blood cells) 4 days prior to the end of the experiment. The splenocytes suspensions were prepared under sterile conditions as the method described by Erwin et al.[16] The viability of separated splenocytes was determined using trypan blue dye (0.1 %) according to Kawabatae and White.[17]

Cellular immunity:
Rosette-forming cells (RFC): Active and total rosette-forming cells were estimated according to Jondal, et al.[18]
Humoral immunity:
Plaque forming cells (PFC): PFC was assessed according to Vos [19] and expressed as the number of plaque forming cells per 10^6 viable splenocytes.

Immunoglobulins level: The serum antibody titres (total Ig) were determined by microtitre procedure and expressed as the log base2 of the reciprocal of the highest dilution giving a visible agglutination to SRBC's. IgG level was recorded as ME resistant using 2-mercaptoethanol (ME). While, IgM level (ME sensitive) equal the difference between the total Ig and ME resistant IgG.[20]

Biochemical studies:
Liver and kidney were homogenized separately in ice cold saline solution (10 % w/v) in a polytron homogenizer (Tekmar tissumizer) for 30 secound. The homogenate was centrifuged at 5000 xg for 30 min at 4 °C using Janetzki cooling centrifuge type K23. The resultant supernatants were used for all different parameters assay except lipid peroxidation and glutathione content were measured in the liver homogenate.
Oxidative stress parameters:
LPO level: LPO process is determined by the thiobarbituric acid (TBA) method, which estimates the malondialdehyde formation (MDA) according to Nair and Turner.\textsuperscript{[21]} The MDA level was measured spectrophotometrically at 532 nm (T80 + UV/ VIS Spectrophotometer PG Instruments Ltd). LPO level is expressed as nmoles MDA / g tissue.

GSH content: GSH as non-enzymatic antioxidant was measured according to Owens and Belcher.\textsuperscript{[22]} Determination of GSH content is based on the reaction of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) with GSH which yield a yellow colored chromophore; 5-thio-nitrobenzoic acid (TNB) with a maximum absorbance at 405 nm. The level of reduced glutathione present in the liver sample was calculated as µg/g tissue.

GPx activity: GPx catalyzes the reduction of hydroperoxides by utilizing GSH. Determination of GPx activity is carried out according to the method of Chiu et al.\textsuperscript{[23]} The enzyme activity is estimated by measurement of the residual reduced glutathione remaining after the action of enzyme with the Ellman’s reagent in the presence of cumene hydroperoxide as a secondary substrate. The specific activity is expressed as mmole/mg protein/min.

Liver function parameters:
AST and ALT activities: were measured by the procedure of Reitman and Frankel\textsuperscript{[24]} using a commercially available kit from Bio-Merieux.

ALP activity: was spectrophotometrically determined according to Bessey, et al.\textsuperscript{[25]} with sodium p-nitrophenyl phosphate as substrate in the alkaline medium at 405 nm.

Kidney function parameters:
Creatinine and urea were spectrophotometrically analyzed using commercial kits from BioScope Diagnostics and ERBA Diagnostics Mannheim GmbH (Germany), according to Kaplan and Pesce\textsuperscript{[26]} and Henry\textsuperscript{[27]} respectively.

Protein Determination:
The total protein content was determined by the method of Lowry et al.\textsuperscript{[28]} using bovine serum albumin as the standard.

Histopathological studies:
Liver and kidney were removed and fixed in 10 % formalin. Then samples were dehydrated by standard procedures and embedded in paraffin, sections approximately 5 μm thick were cut, stained with heamatolin and eosin (H&E) stains and examined by light microscope.\textsuperscript{[29]}

Statistical analysis:
All data were expressed as mean ± standard error (SE). The data was analyzed using one-way analysis of variance (ANOVA) followed by the Student-Newman Keuls test to determine significance between different groups. The criterion for statistical significance was set at p < 0.05. These tests were performed using a computer software CoStat program, version 2.\textsuperscript{[30]}

3. RESULTS
Toxicity study:
Data obtained from the acute toxicity study was illustrated that the acute oral LD\textsubscript{50} value of abamectin against male mice was 38.02 mg/ kg bw with confidence limit ± 0.212.
The in vivo effects of the repeated dose of one-tenth LD₅₀ of abamectin, quercetin (50 mg/kg) and their combination for 14 days on the body and organ weights, hematological, immunological, biochemical parameters as well as histopathological changes were studied.

**Body weight and relative organ weight:**

No mortality occurred in any group throughout the experiment. The physiological status of control and treated animals was noticed as the change in body weight gain and relative organ weights. Results in Table 1 showed that there was significant (p<0.05) decrease in the body weight gain and increase in the relative weights (gm/100 gm body weight) of liver and kidney of animals treated with abamectin compared with control mice by about 1.5 fold. On the other hand, the abamectin did not cause any significant changes in the relative weights of spleen. Treatment with quercetin alone did not cause any significant change in body or organ weights, but alleviated the toxic effect of abamectin.

**Hematological studies:**

The present results revealed that abamectin induced a significant (p< 0.05) decline in Hb concentration, % PCV and RBC count, while WBC count, MCV and MCH values were significant increased. Quercetin alone did not cause any significant changes in these parameters compared to control and it alleviated the negative effect of abamectin (Table 2). On the other hand, there was significant change in the MCHC value in all treatment groups.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Body weight gain (gm)</th>
<th>Relative organ weights (%)(Mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>Control</td>
<td>2.67 ± 0.01 c</td>
<td>5.94 ± 0.19 a</td>
</tr>
<tr>
<td>Quercetin</td>
<td>2.62 ± 0.1 c</td>
<td>6.24 ± 0.28 a</td>
</tr>
<tr>
<td>Abamectin</td>
<td>1.51 ± 0.087 a</td>
<td>8.9 ± 0.16 b</td>
</tr>
<tr>
<td>Quer + Abam</td>
<td>2.21 ± 0.001 b</td>
<td>6.40 ± 0.33 a</td>
</tr>
</tbody>
</table>

Values are expressed as means (5 mice) ± standard errors.
Values in column with different letters are significantly different at p ≤ 0.05.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Experimental Groups (Mean ± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>12.56 ± 0.03 c</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>48 ± 1.15 b</td>
</tr>
<tr>
<td>RBC's (10⁶ cell /µl)</td>
<td>7.77 ± 0.19 b</td>
</tr>
<tr>
<td>WBC's (10³ cell /µl)</td>
<td>10.10 ± 0.08 a</td>
</tr>
<tr>
<td>MCV (Fl)</td>
<td>61.79 ± 0.8 a</td>
</tr>
<tr>
<td>MCH (Pg)</td>
<td>16.19 ± 0.42 a</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>26.21 ± 0.68 a</td>
</tr>
</tbody>
</table>

Values are expressed as means (5 mice) ± standard errors.
Values in row with different letters are significantly different at p ≤ 0.05.
**Immunological parameters:**

There was no significant change between group treated with quercetin and control in all studied immunological parameters (Table 3).

**Splenocytes viability:**

Abamectin significantly reduced cell viability compared to control. While, there is no significant change in groups treated with quercetin or abamectin plus quercetin when compared to control.

**Cellular Immunity:**

**RFC:** Abamectin reduced the percentage of both active and total RFC when compared with control and quercetin alone treatments. In co-administration treatment, the RFC value was increased than abamectin group and reached to control value in the active RFC, while, total RFC was still significant less than control (Table 3).

**Humoral Immunity:**

**PFC:** Abamectin was significantly (p<0.05) reduced the number of PFC/10^6 Splenocytes when compared to the control (41.14 %). Co-administration of abamectin with quercetin group was significantly increased the number of PFC than abamectin alone treatment but still less than the control group (Table 3).

**Immunoglobulins:** It is clear that abamectin decreased the serum immunoglobulins; total Ig , IgG and IgM levels . The quercetin when co-administered with abamectin was normalized the pesticide effect and reached the control levels (Table 3).

**Table (3): Immunological parameters of male mice after 14 days treatment with abamectin (3.8 mg/kg), quercetin (50 mg /kg) and their combination.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Quercetin</th>
<th>Abamectin</th>
<th>Quer + Abam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability (%)</td>
<td>98.00 ± 0.20 b</td>
<td>98.27 ± 0.26 b</td>
<td>94.80 ± 0.89 a</td>
<td>97.92 ± 0.11 b</td>
</tr>
<tr>
<td><strong>Cellular immunity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active RFC (%)</td>
<td>2.54 ± 0.13 b</td>
<td>2.66± 0.20 b</td>
<td>1.92 ± 0.10 a</td>
<td>2.56 ± 0.10 b</td>
</tr>
<tr>
<td>Total RFC (%)</td>
<td>8.76± 1.10 b</td>
<td>8.13 ± 0.47 b</td>
<td>4.49 ± 0.197 a</td>
<td>5.04 ± 0.10 a</td>
</tr>
<tr>
<td><strong>Humoral immunity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFC / 10^6 cell</td>
<td>63.67± 3.18 c</td>
<td>59.67 ± 5.49 c</td>
<td>37.33 ± 2.19 a</td>
<td>50.00 ± 3.46 b</td>
</tr>
<tr>
<td>Ig levels (log2 titer)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Ig</td>
<td>8.33 ± 0.34 b</td>
<td>8.67 ± 0.67 b</td>
<td>5.67 ± 0.34 a</td>
<td>7.33 ± 0.34 b</td>
</tr>
<tr>
<td>Ig G</td>
<td>5.67 ± 0.67 b</td>
<td>5.67 ± 0.67 b</td>
<td>3.67 ± 0.34 a</td>
<td>4.67 ±0.34 ab</td>
</tr>
<tr>
<td>Ig M</td>
<td>2.67 ± 0.34 ab</td>
<td>3.00 ± 0.58 b</td>
<td>2.00 ± 0.58 a</td>
<td>2.67 ± 0.34 ab</td>
</tr>
</tbody>
</table>

Values are expressed as means (5 mice) ± standard errors.

**Biochemical studies :**

Treatment with quercetin alone had no significant effect on all the studied biochemical parameters when compared with control group.
**Oxidative stress parameters:**

Data obtained was indicated that treatment with abamectin caused significant increase in hepatic LPO level and GPx activity, while significant decrease in GSH content compared to control group (Fig.1). Quercetin minimized the toxic effects of abamectin in mice treated with abamectin plus quercetin.

![Figure 1](image.png)

**Liver function parameters:**

Results in Figure 2 showed that hepatic AST, ALT and ALP activities were significantly (P<0.05) increased by abamectin treatment compared with control one. In group 4, quercetin counteracted the toxic effect of abamectin.

**Kidney function parameters:**

The present results also showed that treatment with abamectin caused significant increase (P<0.05) in renal creatinine and urea concentrations compared to control animals (Fig.3). Meanwhile, they were significant decreased in Abamectin plus quercetin group and reached to control value.
Figure 2. Activity of AST (A), ALT (B) and APL (C) in hepatic tissue of male mice treated with abamectin (3.8 mg/kg), quercetin (50 mg/kg) and their combination. Values are expressed as mean ± SE for five animals per group. Different superscript letters indicate statistical significant differences between groups (P < 0.05).

Histopathological examination:
Liver of control and quercetin treated mice showed normal hepatocytes with well-preserved cytoplasm and nucleus in addition to sinusoidal architecture. Liver of abamectin-treated animals showed marked degenerative changes of hepatocytes, congestion and diffuse necrosis of hepatic tissue. Moreover, fibrosis was observed in the portal canal associated with disruption of sinusoids. Quercetin treatment alleviated the lesions caused by abamectin treatment in mice that were represented by few inflammatory cells, infiltration in the portal area, less congested sinusoids, and minimal necrosis (Fig. 4). Concerning the kidney, abamectin alone induced interstitial nephritis, severe degenerative changes in tubules, diffused cellular infiltration and severe congestion of blood vessels, and mild glomerular atrophy, whereas quercetin plus abamectin treatment protected the kidney of mice as evidenced by appearance of mild tubular atrophy, and interstitial inflammatory infiltrate (Fig. 5).
Figure 3. Creatinine (A) and urea (B) level in kidney tissue of male mice treated with abamectin (3.8 mg/kg), quercetin (50 mg /kg) and their combination. Values are expressed as mean ± SE for five animals per group. Different superscript letters indicate statistical significant differences between groups (P < 0.05).

Figure 4. Liver tissue of male mice treated with abamectin (3.8 mg/kg), quercetin (50 mg /kg) and their combination for 14 days using H & E 100 X: Photomicrograph of the liver sections of control (a) and quercetin treated mice (b) showing normal histological appearance of the liver, including, hepatocytes (H), central vein (CV), and sinusoids (S). (c) Section of mice liver treated with abamectin revealed considerable damaged in liver, with the appearance of lymphatic infiltration (LI), focal lytic necrosis (FLN), and sinusoids dilation (DS). (d) Liver section of the mice treated with quercetin + abamectin demonstrated restoration of normal arrangement of hepatocytes, although few damaged hepatocytes were also observed.
4. DISCUSSION

The widespread use of pesticides in public health and agriculture programs has caused severe environmental pollution and health hazards, including cases of severe acute and chronic human poisoning.[31] It is well known that the oral LD50 value of any pesticide is highly dependent on the pesticide and the organism. In this study, the LD50 of abamectin against male mice was 38.02 mg/kg. This result was agreed the data of EPA [32] who reported that the LD50 of abamectin in mice range from 14 to 80 mg/kg, while Eissa and Zidan, 2009 found that it was 18.1 mg/kg in albino rats. The differences in the LD50 values may be due to the presence or absence of P-glycoprotein which is a barrier to tissue entry.[33] He reported that the sensitive CF-1 mice are deficient in P-glycoprotein in the intestinal epithelium and brain capillary endothelium, while insensitive ones showed abundant levels of P-glycoprotein in these tissues and tolerated doses of abamectin at least 50-fold the minimum toxic dose in the sensitive group. All avermectins interfered with the transport activity of P-glycoprotein, which acts as a transmembrane protein, transporting some drugs into and out of cells. Animals showing decreased P-glycoprotein level show greater bioavailability of a drug after oral administration and accumulate greater levels of drugs in their CNS tissue.[33]

In toxicological studies body and organ weights of animals are important criteria for the evaluation of toxicity.[34] The body weight of abamectin treated mice was significantly (p< 0.05) lower than that of the control group. This may be attributed to a decreased food intake (anorexia or food avoidance), poor food palatability or increased degradation of lipids and protein due to treatment-related toxicity.[35] Similar results have been found in animals exposed to avermectin compounds (abamectin, emamectin benzoate).[36, 37] Results showed that there is a significant increase in the relative liver and kidney weights in mice exposed to abamectin. This increase is parallel with the data of Aly and EL-Gendy [38] in rabbit. The enlargement may be due to the accumulation of abnormal amounts of fat, predominately triglyceride in the parenchymal cells. Triglyceride accumulation is a result of an imbalance between the rate of synthesis and the rate of release of triglyceride by the parenchymal cells into the systemic circulation.[39]
Abamectin treatment resulted in a significant (p< 0.05) decline in the values of Hb, PCV and RBC’s. The reduction of RBC may be attributed to the failure to supply the blood circulation with cells from hemohepatic tissue, since the liver has an important role in the regeneration of erythrocyte and the possible destructive effect on erythrocyte by the toxicants. Reduction in hemoglobin content can be related to the decreased size of red blood cells, the impaired biosynthesis of hem in bone marrow or the increased rate of destruction / reduction in the formation rate of total erythrocyte count. Increase fragility and progressive destruction of RBC’s due to binding of free radicals produced by abamectin could be another reason for decreased hematological values. One of the molecular mechanisms of the toxicity of some pesticides seems to be lipid peroxidation; consequently, these compounds can disturb the biochemical and physiological functions of the erythrocyte. The susceptibility of red blood cells to oxidative damage is due to the presence of polyunsaturated fatty acid, hem iron and oxygen, which may produce oxidative changes in erythrocytes. The increase in WBC’s count may indicate activation of the animal’s defense mechanisms and immune system or due to inflammation caused by pesticide general toxicity. The increase in MCV and MCH values in treated animals may be due to that red blood cells are larger than normal (macrocytic). Moreover, increase MCHC value (hyperchromia) is seen in conditions where the hemoglobin is more concentrated inside the red blood cells.

The immune system is important for defense against a variety of insults. It is a highly evolved system and is distributed throughout the body. The complex nature of the immune system with its multiple humoral and cellular components makes it an easy target for many drugs and chemicals. In the present study, we select RFC as a marker of cellular immunity and IgG, IgM and PFC as markers of humoral immunity. The results showed that abamectin reduced both cellular and humoral immunity; this may be due to the directly inhibited antibody synthesis, caused by subacute or chronic stress situation which was responsible for the reduced titers. Also, the decrease in antibody titer and PFC in mice treated with abamectin may be due to the decrease of total number of circulating B-cells, direct suppression of its functional capacity or degeneration of B-lymphocytes in spleen. On the other hand, abamectin may be metabolically converted to a reactive electrophilic derivative and once it was formed, it may bind to critical sites on DNA and / or other molecular targets that are important in the PFC response. Our results are in agreement with those of Kawabatae and White, Riahi et al. who reported similar marked depression of cellular and humoral immunity in animals treated with a variety of pesticides.

Pesticides can induce oxidative stress by generation of free radicals that might cause lipid peroxidation, alternations in membrane fluidity, DNA damage and finally carcinogenic effects. In vertebrates, the liver is a highly metabolically active organ, with a high activity of antioxidants and associated enzymes, so that it is the main organ responsible for detoxification of xenobiotics. In the present study, abamectin treatment to mice indicated a marked increase in the hepatic LPO and decrease in GSH level. This result coincides greatly with Verma et al., Gultekin et al., Khan and Kour, Ashar Waheed and Muthu Mohammed. LPO is the process of oxidative degradation of polyunsaturated fatty acids (PUFA) and its occurrence in biological membranes causes impaired membrane function, structural integrity, decrease in membrane fluidity and inactivation of a several membrane bound receptors and enzymes. The present result strengthens this hypothesis and suggests that induction of oxidative stress is perhaps the central mechanism by which such tested pesticide exerts its cytotoxic effects. GSH is an important naturally occurring antioxidant, which prevents free radical damage and helps detoxification by conjugating with chemicals and also, it acts as an essential cofactor for antioxidant enzymes. Under oxidative stress, GSH is consumed by GSH related enzymes to detoxify peroxides produced due to increased lipid peroxidation. The biological function of GPx is to reduce lipid...
hydroperoxides conversion to their corresponding alcohols and to reduce free hydrogen peroxide reaction.\cite{49} GPx activity was significantly increased in mice treated with abamectin. This result is in agreement with many authors.\cite{50, 55} Liver is often the primary target for the toxic effects of xenobiotics. It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver.\cite{56} Therefore, liver can be used as an index for the toxicity of xenobiotics. Hence, the activity of some enzymes; AST, ALT and ALP, is representing liver function. Administration of abamectin significantly increased the levels of AST, ALT and ALP; these findings are in coincidence with those reported by Eissa and Zidan\cite{41}, Abd-Elhady and Abou-Elghar.\cite{57} The disruption of transaminases from the normal values denotes biochemical impairment and lesions of tissues and cellular function because they are involved in the detoxification process, metabolism and biosynthesis of energetic macromolecules for different essential functions.\cite{58} In the case of liver injury resulting from hepatotoxicity, cellular contents are released, thereby increasing the level of liver enzymes above the normal threshold. In the present study, the increased levels of AST and ALT may be due to hepatotoxicity causing permeability alterations and leakage of lysosomal enzymes enhancing the release of enzymes.\cite{56} The elevation of ALP activity in mice treated with abamectin is in accordance with the findings of Rahman et al.\cite{59} who reported that the increase in the activity of ALP or ACP might be due to the necrosis of liver, kidney and brain. Creatinine and urea are useful in early deduction of nephrotoxicity induced by exogenous compounds. These parameters are used as index of renal damage in living organisms.\cite{60} Our study also revealed the obvious significant increase in creatinine and urea concentrations with the subacute abamectin administration. Similar findings were demonstrated by Eissa and Zidan\cite{41}, Abd El-Hady and Abou Elghar.\cite{57} Elevation of creatinine and urea concentrations of treated animals may be attributed to reduction in glomerular filtration in the kidney and also reflect dysfunction of the kidney tubules.\cite{41} Histological changes provide a rapid means for detecting the effects of pesticides in various animal tissues and organs.\cite{36} The light microscopic investigations showed many histological abnormalities in the liver and kidney of abamectin exposed mice. Clinical chemistry and histopathological evaluations are commonly used methods for detecting organ specific effects related to chemical exposure.\cite{61} Alterations of ALT, AST and ALP activity could be due to necrotic changes of hepatic tissue that appears in histopathological examination like congestion, haemorrhages, fibrosis in the portal canal associated with disruption of sinusoids due to decreased free radical (O^{-2}) scavenger formation. The change in the biochemical parameters of kidney was also correlated closely with the histopathological changes such as marked hemorrhage, congestion, and other degenerative.\cite{57} Our data found amelioration of the adverse effect of abamectin on ALT, AST, ALP, creatinine and urea when quercetin was co-administrated with abamectin.\cite{62} The hepatoprotective and nephroprotective effect of quercetin might be due to inhibition of oxidative stress.\cite{63} In general, our data indicated that abamectin has cytotoxic effects on male mice evidenced by change the blood picture, increase of oxidative stress, humoral and cellular immunity and function of liver and kidney parameters as well as histopathological changes. However, the presence of quercetin with abamectin alleviated its harmful effects on most of all the above measured parameters and corrected level of these parameters was observed likely to near normal values of the control or quercetin alone. Our data are in agreement with Gergouri et al.\cite{63}, Ashar Waheed and Muthu Mohammed\cite{52} who found that quercetin has protected animals from pesticides induced harmful effects. From the present results, it can be concluded that exposure of animals to abamectin is capable of inducing free radicals, which making hazardous alterations in mice. The free radicals will seek out an electron to regain their stability. Antioxidants are known to reduce oxidative radical induced reactions. Quercetin is an excellent source of electrons; therefore, it can donate electrons to free radicals such as hydroxyl and superoxide and quench their reactivity.\cite{52, 63} Quercetin supplementation has hepato- and nephro- protective effects in abamectin induced liver and kidney toxicity. Quercetin can scavenge free radicals and /or
inhibit their formation, so it can protect the DNA of the cells from damage. Quercetin combats the effects of many toxins, including pesticides and heavy metals and also, fights off these pollutants by stimulating liver enzymes that detoxify body.

5. CONCLUSION
In conclusion, the present study shows that despite abamectin is a natural fermentation product of the soil-dwelling actinomycete (*Streptomyces avermitilis*), it causes at the repeated dose of 3.8 mg/kg for 14 days significant changes in the hematological, immunological and biochemical parameters, besides the histological changes of male mice. In addition, quercetin supplementation significantly protected the adverse effects of abamectin. It is a wise step to take care to avoid toxins such as pesticides as much as possible and to enrich our diets with life giving antioxidants such as quercetin especially in our increasingly toxic world. Thus, sufficient dietary intake of quercetin by individuals who regularly come in contact with pesticides is beneficial in combating the adverse effects of abamectin.

6. REFERENCES
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