Histological and cytogenetic effects of Acetamiprid on male albino mice

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ABSTRACT

The present study aimed to investigation of acetamiprid effects on histological and immunological aspects in male albino mice. Eighteen male albino mice aged (6-7 weeks) were divided into three groups each having (6) healthy mice. The first group orally administrated with distilled water while the second and third groups were orally administrated with 10mg/ml and 20mg/ml respectively of acetamiprid (LD50=200mg/kg) (0.1ml) daily for two weeks. The parameters of evaluations were included White blood cell count (WBC), Differential count (DC), Micronucleus formation in bone marrow and histological sections for two organs (liver and spleen). The study suggested that acetamiprid 20mg/ml significantly affected whether histological or immunological and it was considered to be toxic dose of acetamiprid in albino mice.

INTRODUCTION

Pesticides are the chemical formulation increasingly used in agriculture, animal husbandry and public health operation to kill the insects, weeds and fungus and to get rid of insect transmitted diseases [1]. These pesticides are toxic not only to insects and pests but at different levels to animals and human beings [2]. It is clear from other animal studies that many chemicals, including pesticides, can alter their immune system either morphologically or functionally [3,4]. An immunomodulation caused by the action of contaminants could subsequently reduce the ability of these animals to defend themselves against invading pathogens [5,6]. Acetamiprid, a member of the neonicotinoid [7], and the neonicotinoids are the newest major group of insecticides, which includes acetamiprid, imidacloprid, clothianidin, dinotefuran, nitepyrar, thiacloprid, and thiamethoxam [8]. It is highly effective for the controlling aphids, beetles, moth, leathnapper, pests on crops and leafy vegetables, along with fleas infesting livestock and pet animals [9]. It is a systemic insecticide with translinar action which has a contact and stomach action. Moreover, acetamiprid being highly water soluble indicates a high potential for the compound to leach in soil or to run off in surface water [10]. Furthermore there are many studies indicated the increased use of pesticide resulted in toxicity in different species and could affect various functions like neurological, hematological, biochemical and reproductive function etc. in the body. These studies on toxicological aspect of insecticides are always useful for the rational treatment and prediction of risk of toxicity [11]. Thus in present study we used mice to focused on some immunological and pathological changes as an indirect exposure in human and mammals in general.

MATERIALS AND METHODS

Animals: Albino male mice (18 animals at age 6-7 weeks) were used and distributed into three groups, each with 6 mice. The first group was normal controls, which were administrated with 0.1 ml of distilled water. The second group included mice orally administrated with Acetamiprid (10mg/ml). The third group was orally administrated with Acetamiprid (20mg/ml). Second and third groups were orally administrated with 0.1ml from both concentrations for 14 day.

Experimental design: Albino male mice were weighted before orally administration, and then weighted weekly after oral administration with pesticides. On day 14 six mice of each groups were sacrificed and organs (liver and spleen) were weighted and prepared for tissue
sectioning [12]. Some laboratory evolutions were examined, which was white blood cell count (WBC), differential count (DC) [13] and micronucleus formation [14]. The micronucleus index was obtained using the following equation:

\[
\text{Micronucleus Index (micronucleus/cell)} = \left( \frac{\text{Number of Micronuclei}}{\text{Total Count of polychromaterythrocytes}} \right) \times 100
\]

Statistical analysis: Data are presented as mean ± standard error (S.E.), and differences between means were assessed by ANOVA-one way test followed by Duncan test, in which P ≤ 0.05 was considered significant. The analyses were carried out using the statistical package SPSS (Statistical Package for Social Sciences) version 13.

RESULTS AND DISCUSSION

The body weight was significantly (P ≤ 0.05) decreased in mice treated with acetamiprid after 14 days as compared with controls in both doses (Table 1). While the results showed slightly increased in weights of organs liver and spleen in mice treated with acetamiprid (10 and 20mg/ml respectively) (Table 2), as compared with controls (1.45±0.05 and 1.47±0.04 respectively)(0.18±0.01 and 0.19±0.02 respectively), as well as the mice suffering from some changing in behaviors such fatigue, loss of appetite, loss of activity and drowse appeared after a week on oral administration with acetamiprid. The increasing in the liver weight may be attributed to the presence a large number of hepatocytes suffering from vaculation / fatty change and this results are accordant with the pathological changes in the liver histology and agreement with study of [15], that deals with the effect of a mixture of five pesticides in the liver histology and agreement with study of [15], [16] thus decreased body weight in rats were observed in a 90-day study [17],or to the formation of some bundles of fibers[18]. Because of the decrease of the final body weight in the high dose through the time of administration , therefore observed an increase of organs weights[19].

Table (1): Effect of Acetamiprid (mean±standard error) on weight of male albino mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/ml</th>
<th>Before administration</th>
<th>After1Week of administration</th>
<th>After2Weeks of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D.W</td>
<td>19.23±0.61C</td>
<td>20.20±0.33B</td>
<td>21.18±0.62A</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>22.47±1.75C</td>
<td>20.87±0.75B</td>
<td>19.70±0.60A</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>21.93±0.70B</td>
<td>21.65±0.59B</td>
<td>19.69±0.55A</td>
</tr>
</tbody>
</table>

Different letters represent significant difference (P ≤ 0.05) between means of rows, while similar letters represent no significant difference (P > 0.05) between these means (Duncan test).

Table (2): Effect of Acetamiprid (mean±standard error) on Weight of Organs (Liver and Spleen) in male albino mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/ml</th>
<th>Liver mg/mouse</th>
<th>Spleen mg/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D.W</td>
<td>1.28±0.02B</td>
<td>0.12±0.01B</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>1.45±0.05A</td>
<td>0.18±0.01A</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>1.47±0.04A</td>
<td>0.19±0.02A</td>
</tr>
</tbody>
</table>

Different letters represent significant difference (P ≤ 0.05) between means of columns, while similar letters represent no significant difference (P > 0.05) between these means (Duncan test).

Significant increased in white blood cells count (total and differential) specially at dose (20mg/ml) (8.86±0.29, 5.66±0.26, 2.70±0.18, 0.27±0.02 and 0.08±0.01 respectively) (Table 3). Also the same results was observed in micronucleus forming after the exposure periods of (7 and 14days ) that determine the genotoxic and cytotoxic effects of acetamiprid (Table 4). The results show that oral administration of the high does (10 and 20 mg/ml) of Acetamiprid cause a significant increase when compared with control. These results were in agreement with those described by Jain et al.[20], who found the same after intraperitoneal administration of a sub acute dose of imidacloprid (neonicotinoid pesticide) in adult male rats. This response to pesticide administration according to Rivarola and Balegno(1991) could be attributed to changes in protein metabolism and their synthesis in the liver. As well as to the damaging effect of insecticides on liver cells[21].

Acetamiprid induced the more chromatid breaks than the other structural chromosomal aberrations. Therefore, micronucleus (MN) can be formed from acentric chromosomal fragments. And also, due to the chromatid break was the most observed aberration, this pesticide may be acted in the late or G2 phase of the cell cycle [22]. The increasing of micronucleus (MN) formation due to This pesticide indicates the clastogenicand DNA damaging potential of it. [23]. This insecticide showed different pathological lesions in the liver tissue. It is
clear that liver tissues are markedly responded to the adverse effect of (acetamiprid) and displayed marked histological changes. The liver is the centre detoxifying any foreign compounds entering the body. So, it uniquely exposed to a wide variety of exogenous and endogenous products. These include environmental toxins and chemicals present in food or drinking [24,25] reported that liver of treated rats with diflubenzuron, cypermethrin and fenitrothion showed different phases of degenerative changes in the form of cloudy swelling, hydropic degeneration, chromatolysis, pyknosis, fatty degeneration. In the same respect, data obtained by [26] showed that 1/10LD 50 of lufenuron caused venous congestion in the liver, focal necrosis of hepatocytes in the portal and periportal areas. Many of the hepatocytes were palestained and a few exhibited early vacuolation.

Table (3): Effect of Acetamiprid (mean± standard error) on white blood cell count (Total and Differential) in male albino mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/ml</th>
<th>White Blood Cell count × 10³</th>
<th>Cell /Mm³ blood and differential</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>W.B. C count</td>
<td>Lymphocytes</td>
</tr>
<tr>
<td>1</td>
<td>D.W</td>
<td>7.67± 0.30B</td>
<td>4.42± 0.33C</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>7.38± 0.48B</td>
<td>5.27± 0.26B</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>8.86± 0.29A</td>
<td>5.66± 0.26A</td>
</tr>
</tbody>
</table>

Different letters represent significant difference (P ≤ 0.05) between means of columns, while similar letters represent no significant difference (P > 0.05) between these means (Duncan test).

Table (4): Effect of Acetamiprid (mean± standard error) on Micronucleus forming in bone marrow cell of male albino mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose mg/ml</th>
<th>Micronucleus\cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>D.W</td>
<td>0.0066±0.005C</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>0.0074±0.004B</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>0.0086±0.004A</td>
</tr>
</tbody>
</table>

Different letters represent significant difference (P ≤ 0.05) between means of columns, while similar letters represent no significant difference (P > 0.05) between these means (Duncan test).

Histopathological investigation of sections of liver of the treated mice showed After 7 days of administration of both doses. Congestion and Mild hemorrhages (Figure 1, 2, 3)
Figure (4): Photomicrographs of a section of the liver of albino mice after the administration of acetamiprid (10,20mg/ml) day7, showing disturbed hepatic lobule in the structure of the hydropic degeneration in the hepatocytes and dilation of the hepatic sinusoid. H&E.

On the other hand, sections of liver of some treated mice after 14 day of administration for both concentrations showed vacuoles in the cytoplasm of the hepatocytes and focal necrosis of hepatocytes in the portal and periportal areas (Figure 5and6).

Figure (5): Photomicrographs of a section of the liver of albino mice after the administration of acetamiprid (10,20mg/ml) day14, showing vacuoles in the cytoplasm of the hepatocytes and focal necrosis of hepatocytes in the portal and periportal areas. H&E.

Figure (6): Photomicrographs of a section of the liver of albino mice after the administration of acetamiprid (10,20mg/ml) day14, showing vacuoles in the cytoplasm of the hepatocytes and focal necrosis of hepatocytes in the portal and periportal areas. H&E.

Hepatocytes showed pyknotic or karyolytic nuclei and the cytoplasm showed pale stain (Figure 7,8).

Figure (7): Photomicrographs of a section of the liver of albino mice after the administration of acetamiprid (10,20mg/ml) day14, showing vacuoles in the cytoplasm of the hepatocytes and focal necrosis of hepatocytes in the portal and periportal areas. H&E.

Figure (8): Photomicrographs of a section of the liver of albino mice after the administration of acetamiprid (10,20mg/ml) day14, showing vacuoles in the cytoplasm of the hepatocytes and focal necrosis of hepatocytes in the portal and periportal areas. H&E.

Oral administration of Acetamiprid induces marked alterations in the histology of the spleen. Animals treated with (10,20mg/ml) of insecticide for 7, days showed a red pulp congested with red blood cells and hemorrhagic areas (Figure 9and10).
Figure (9,10): Photomicrographs of a section of the spleen of albino mice after the administration of acetamiprid (10,20mg/ml) day 7, showing a red pulp congested with red blood cells and hemorrhagic areas. H&E.

Spleen sections from animals treated with (10,20mg/ml) of insecticide for 14 days showing small nests of pyknotic nuclei and fibroblasts(Figure 11,14), small bundle of fibers(Figure 12,13).

Figure (11,12): Photomicrographs of a section of the spleen of albino mice after the administration of acetamiprid (10,20mg/ml) day 14, showing small nests of pyknotic nuclei and fibroblasts. H&E.

Figure (13,14): Photomicrographs of a section of the spleen of albino mice after the administration of acetamiprid (10,20mg/ml) day 14, showing small bundles of fibers. H&E.
Our data showing the same response in spleen compared with the spleens of rats treated with 1/100 LD50 of IC insecticide showed many changes that may be due to the toxicity of imidacloprid. Such changes included lymphocyte depletion in the white pulp associated with many aggregates of pyknotic cells. In addition, the walls of the central arterioles ruptured and showed increasingly narrow lumens. Major changes in the red pulp included increases in the numbers of macrophages, neutrophils and nests of pyknotic cells. Similar spleen changes were induced by Balani [27], and all of these changes may be attributed to a loss of infiltration efficiency. The detection of pyknosis in the spleen may be related to an increase in T cell susceptibility to apoptosis, which may be an important mechanism of autoimmune diseases and immune senescence [28]. Examination of sections of spleen of the treated mice showed congestion and heamorrhagic areas. The same with El-Hawashy and Khedr (2001) who showed congestion, degeneration and large lymphoietic follicles in spleen tissues of albino rats treated with calciferol rodenticide.

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