Effect of dietary exposure to diazinon on different organs and hematological parameters of rabbit

MA Hassan, T Rahaman, KA Ferdous, MNH Parvez and MS Islam

Abstract

The irrational use of diazinon in our country can play a crucial role in the occurrence of many diseases of animals and human. The present study was conducted to investigate the alteration of gross & microscopic study of various organs, hematological parameters of rabbit as effect of insecticide (Diazinon). A total number of 16 male rabbits were assigned into four group. Control group received only basal diet and the other groups T1, T2 and T3 received diazinon with supplemented feed at a dose level of 15, 30 and 45mg/kg feed respectively. Remarkable decreased in weight gain in T3 group. Diazinon caused elevation of SGPT/ALT (P<0.01), serum creatinine level attributed to pathological lesions. Histopathological lesions in liver included cirrhosis and necrosis in only (T2 & T3) treated groups. In kidney, glomerulus was filled with reactive cells in group T1. There was slight loss of normal histological structure of pancreas in group T1 rather than group T2 & T3.

Keywords: Rabbit, diazinon, different organs, hematological parameters

1. Introduction

Livestock plays a significant role in the economy of our country. The livestock sector plays an important role for the major supply of protein of our country. Country’s 25 percent people are directly engaged in livestock sector and 50 percent people are partly associated in livestock production. The contribution of livestock sub-sector to the total GDP was 2.95 percent which was estimated about 17.32 percent GDP to agriculture [6]. According to the department of livestock services about 36 percent of the total animal protein comes from the livestock products in our daily life. However, this production of animal protein is quite insufficient to fulfill the demand of overflown population in the country. Many people especially poor people cannot take sufficient amount of protein for maintaining their healthy body due to high cost of meat. It is the result of over population and improper utilization of our livestock sector. To fill up this gap of animal protein availability, diversified farming for example rabbit farming can play a significant role. Rabbit is an excellent source of animal protein because it gives birth 5-7 times in one year and in each parturition they give birth near about 7-8 offspring maximum and minimum 3-4 offspring. Within 5-7 months they become sexually mature and can be slaughtered for animal protein. This protein source is very healthy for human body due to the low fat concentration.

Rabbit farming is an urgent demand in our country, but it could be the source of public health hazard in certain cases. Contamination of animal feed with environmental pollutants (xenobiotics) is one of the potential health hazards. Animal feeds are made from different raw materials, such as plant and animal source as well as pharmaceutical and industrial origin. Insecticide is one of the most common xenobiotics which are being uncontrolled used in crop field and thereby contaminates the animal foods [4]. Insecticide provides adverse effects on human health through disrupting multiple cellular communication and normal physiological action [15]. The indiscriminate release of insecticide into the soil, water and plant is a major health concern worldwide, as they cannot be broken down to non-toxic forms and therefore have long-lasting effects on the ecosystem. Insecticides are organic compounds that are deliberately introduced into the environment to control selected organisms. Many of insecticide are toxic even at very low concentrations and are not only cytotoxic but also carcinogenic and mutagenic in nature. Agricultural and domestic insects are controlled by pesticides throughout the worldusing organophosphorus ins. Contact with organophosphorus
pesticides is an important health problem for agricultural workers \[23\]. Some of these are highly toxic for mammals \[33, 13\], bird and fishes \[37, 35\]. For the control of flies, lice, and other insect pests of ornamental plants and food crops, diazinon is widely used in the world \[24\]. Due to extensive use of diazinon, its residues have been detected in foodstuffs designed for human consumption \[24\]. The toxic effects of diazinon on animals were studied \[1, 34, 9\] and reported that diazinon inhibits acetylcholinesterase activity and other organic functions. Diazinon was also found to lead to alterations in hematological parameters in male rats \[5, 27\] and hepatotoxicity in rats \[28\]. In a previous study \[17\] showed histopathological changes in different organs of rats by diazinon.

Recently, we have noted that a number of people suffering from various liver and kidney related diseases. Therefore the proposed research aims to investigate the effect of diazinon contaminated food on histopathological changes in different organs of the body and biochemical alteration in rabbit.

2. Materials and methods

The average weight range of the male rabbit used in the study was 1250-1300 gm. The experiment was carried out in the Laboratory of Anatomy and Histology, Hajee Mohammad Danesh Science and Technology University during the period from January to June 2018. The rabbit were free from any congenital disorders and remarkable disease that may cause any problem in the study. The chemical diazinon (trade name-rizinon) used in this experiment were obtained from Polyvar Agro Industries Limited, Bangladesh. Sixteen male rabbits were randomly allotted into four groups T0, T1, T2 and T3. The rabbit were kept under observation for 3 days with basal diet before starting of treatment with diazinon. The rabbit of group T0 were kept as healthy control provided with only basal diet (cabbage, napier grass, broiler grower from CP feed), rabbits of group T1 were given with diazinon powder @15 mg/kg feed, group T2 @30 mg/kg feed and group T3 @45 mg/kg feed with basal diet. The rabbit were supplied with fresh drinking water adlibitum. Blood was collected from the rabbit prior to the treatment start and recorded the random blood sugar (RBS), serum creatinine and serum glutamic pyruvic transaminase (SGPT).

All rabbits during the treatment period were examined daily for abnormal physical and behavioral changes as well as mortality (if any) due to diazinon toxicity. Initial body weight of individual rabbit on first day of experiment was recorded. Subsequently body weights were recorded at every three days interval up to 45 days for each group. At the end of the experiment, 3 rabbits from each of treatment groups including control were randomly selected and slaughtered after 12h of fasting to collect the viscera (liver, kidney, heart and stomach) and muscle to find the gross changes of those organs. Blood samples were collected from the ear vein before slaughtering into labeled EDTA bottles from each group of rabbit for blood analysis including Serum Glutamic Pyruvic Transaminase (SGPT)/ Alanine Transaminase (ALT), Serum creatinine and Random blood sugar.

For histopathological study the collected samples were preserved for fixation in the bouin’s fluid for 24 hours. The tissues were then dehydrated by using ascending graded of alcohol (70%, 80%, 90%, 95%, 100% and 100%) and kept for one hour in each grade of alcohol. The tissues were then transferred to the xylene-1 and xylene-2 each for ninety minutes. Then the tissues were infiltrated in the liquid paraffin at 60ºC temperature for ninety minutes and repeated again. Finally the tissues were embedded in paraffin and paraffin blocks were made. The paraffin blocks were cut at 6 µm thickness using microtome machine (Mu 509, Euromex, Japan). After sectioning of paraffin block, the slices were floated on warm water in a water bath at 45 ºC for stretching. The sections with glass slides were stained with Hematoxylin and Eosin (H & E) stain for general histological study. Observations of the slides were done using a light microscope and photographs were taken with an automatic photo micrographic system.

Data were expressed as mean ± standard error (SE) and analyzed using one way Analysis of Variance (ANOVA) followed by Duncan’s test as a post-hoc test using IBM SPSS Statistics 20.0 software package and the chart was created by Microsoft Excel 2007 software. Results were considered to be statistically significant when P values are less than 0.01 (P<0.01).

3. Results

In this experiment, all the treatment groups supplied with various levels diazinon showed significant differences in body weight, gross changes, microscopic changes and hematological parameters.

3.1 Clinical findings

No apparent clinical signs of diazinon toxicity were observed in rabbit of any treatment groups for the first 7 days. After 7 days, clinical symptoms (mild depression, vomiting, reduced feed intake, dullness, and gastrointestinal signs such as diarrhea & abdominal tenderness, difficult or labored breathing, lack of appetite for food or water, lethargy, mouth irritation, weakness. Mortality was observed only in T3 (diazinon 45 mg/kg feed) experimental group.

3.2 Body weight

During the experiment, supplemental dietary diazinon significantly reduced body weight (P<0.01) compared to control (Table1). Decreased body weight was found throughout the experimental period in diazinon treated rabbit and the rate of reduced body weight was proportional to consumption of diazinon. At 43rd day of treatment group T1 (diazinon 45 mg/kg feed) showed lowest body weight (1022.66±22.16gm) whereas group T2 (30mg/kg feed), group T3 (diazinon 15mg/kg feed) and control T0 (no diazinon) had body weight (1193.66±44.34gm), (1207.00±26.0 gm) and (1355.00±25.63 gm) respectively.

3.3 Gross findings

At the end of experiment, postmortem examination grossly showed no major change in pancreas, but diffuse congestion and enlargement of kidney, pale color and enlargement of liver (hepatomegaly), splenomegaly, congestion in stomach and intestine (Fig. 3), (Fig. 10) & (Fig.7) in the diazinon treated rabbit. Gross examination of control group (group T0) revealed normal liver, spleen, heart, lung and kidney (Fig. 1), (Fig. 2) & (Fig.5).

3.4 Microscopic findings

Upon microscopic observation, pancreas, liver, and kidney of rabbit treated with diazinon showed varying degree of vascular changes like infiltration of inflammatory cells, degenerative changes including necrosis and fibrosis of...
hepatocytes, loss of normal architecture of parenchyma of pancreas & connective tissue proliferation in the tubular and peritubular structure of kidney were also reported.

Group T1 (15mg/kg feed) had slightly loss of regular pattern of hepatic cord (Fig. 16), also loss of normal histological structure of pancreas (Fig. 12) and kidney (Fig.20). Microscopic examination of kidney of group T1 showed no remarkable change in kidney tubules (proximal and distal tubular and peritubular structure and fatty degeneration & of pancreas (Fig. 14); connective tissue proliferation in interlobular septa and intralobular parenchyma langerhans of pancreas (Fig. 13), some infiltration of reactive & inflammatory cell with conn ective tissue proliferation in

Group T3 (45mg/kg feed) showed highly disarrangement of kidney tubules also shown (Fig. 21). Rabbit exposed to diazinon showed no significant increase or decrease in random blood sugar (P<0.01) at 43rd days as compared to the control group (Table 2). The random blood sugar was found normal in group T1 (11.24±0.14 mol/l), T2 (12.59±0.83 mol/l) and T3 (11.55±0.62 mol/l) compared to control T0 (11.18±0.82 mol/l). Diazinon treated rabbits had shown a significant increase in Serum creatinine (P<0.01) at 43rd days as compared to the control group (Table 3). Increase serum creatinine was found in group T3 (1.28±0.13mg/dl), T2 (0.99±0.05mg/dl) and T1 (0.90±0.05mg/dl) compared to control T0 (0.73±0.12mg/dl) received normal feed. On the other hand, diazinon treated rabbits had shown significantly higher SGPT/ALT (P<0.01) concentrations at 43rd days compared to the control group (Table 4). Highest SGPT was found in group T3 (14.23±1.88u/l) followed by group T2 (12.59±1.88u/l) and group T1 (10.05±0.17u/l) against to control T0 (8.53±0.44u/l).

### Table 1: The effects of different levels of diazinon on body weight (gm) of rabbit from 0 days to 43rd days of experiment

<table>
<thead>
<tr>
<th>Days</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
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<tbody>
<tr>
<td>D0</td>
<td>1250.00±28.86</td>
<td>1250.00±28.86</td>
<td>1250.00±28.86</td>
<td>1250.00±28.86</td>
</tr>
<tr>
<td>D3</td>
<td>1255.00±30.41</td>
<td>1250.33±28.57</td>
<td>1250.00±28.86</td>
<td>1287.00±56.7</td>
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<tr>
<td>D7</td>
<td>1257.66±30.60</td>
<td>1248.66±28.89</td>
<td>1243.33±42.71</td>
<td>1278.33±48.6</td>
</tr>
<tr>
<td>D10</td>
<td>1258.66±30.60</td>
<td>1248.00±28.93</td>
<td>1227.33±42.21</td>
<td>1275.33±47.7</td>
</tr>
<tr>
<td>D13</td>
<td>1261.00±31.24</td>
<td>1246.66±29.05</td>
<td>1227.33±42.77</td>
<td>1270.00±48.0</td>
</tr>
<tr>
<td>D16</td>
<td>1264.66±30.71</td>
<td>1243.66±27.47</td>
<td>1223.33±44.34</td>
<td>1276.66±47.0</td>
</tr>
<tr>
<td>D19</td>
<td>1267.33±31.12</td>
<td>1241.33±29.09</td>
<td>1223.33±43.72</td>
<td>1130.00±13.2</td>
</tr>
<tr>
<td>D22</td>
<td>1275.00±35.47</td>
<td>1238.00±28.93</td>
<td>1222.33±43.91</td>
<td>1107.33±6.3</td>
</tr>
<tr>
<td>D25</td>
<td>1280.66±30.87</td>
<td>1232.66±27.42</td>
<td>1219.33±42.41</td>
<td>1104.66±2.6</td>
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<tr>
<td>D28</td>
<td>1282.33±30.20</td>
<td>1228.33±27.4</td>
<td>1216.33±41.2</td>
<td>1101.66±1.6</td>
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<tr>
<td>D31</td>
<td>1285.33±30.39</td>
<td>1223.33±28.38</td>
<td>1211.66±40.96</td>
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<tr>
<td>D34</td>
<td>1288.66±31.13</td>
<td>1217.00±26.31</td>
<td>1205.00±43.09</td>
<td>1060.33±29.16</td>
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<tr>
<td>D37</td>
<td>1291.33±30.80</td>
<td>1213.00±27.73</td>
<td>1201.33±43.76</td>
<td>1056.66±25.8</td>
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<tr>
<td>D40</td>
<td>1297.33±32.24</td>
<td>1210.33±26.90</td>
<td>1197.33±43.91</td>
<td>1054.33±22.7</td>
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<tr>
<td>D43</td>
<td>1335.00±25.63</td>
<td>1207.00±26.0</td>
<td>1193.66±44.34</td>
<td>1022.66±22.16</td>
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</table>

Means on the same row with different superscripts are significantly different (P<0.01). SE: Standard Error NS: Non-significant and S: Significant at 1% level

### Table 2: Random Blood Sugar (RBS) of rabbit at 43rd days fed varying levels of diazinon

<table>
<thead>
<tr>
<th>Parameters [Random blood sugar or RBS (mol/l)]</th>
<th>Various treatment groups showing mean ± SE values</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st test (D0)</td>
<td>10.73±0.3</td>
<td>10.65±0.22</td>
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<tr>
<td>2nd test (D22)</td>
<td>10.92±0.43</td>
<td>11.66±0.52</td>
</tr>
<tr>
<td>3rd test (D43)</td>
<td>11.18±0.82</td>
<td>11.55±0.62</td>
</tr>
</tbody>
</table>

### Table 3: Serum creatinine of rabbit at 43rd days fed varying levels of diazinon

<table>
<thead>
<tr>
<th>Parameter [Serum creatinine (mg/dl)]</th>
<th>Various treatment groups showing mean ± SE values</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st test (D0)</td>
<td>0.73±0.09</td>
<td>0.76±0.09</td>
</tr>
<tr>
<td>2nd test (D22)</td>
<td>0.70±0.06 *</td>
<td>0.83±0.06 *</td>
</tr>
<tr>
<td>3rd test (D43)</td>
<td>0.73±0.12 *</td>
<td>0.90±0.05 b</td>
</tr>
</tbody>
</table>

### Table 4: SGPT of rabbit at 43rd days fed varying levels of diazinon

<table>
<thead>
<tr>
<th>Parameters [SGPT(u/l)]</th>
<th>Various treatment groups showing mean ± SE values</th>
<th>Level of Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st test (D0)</td>
<td>7.55±0.89</td>
<td>7.28±0.99</td>
</tr>
<tr>
<td>2nd test (D22)</td>
<td>7.65±0.93 *</td>
<td>8.80±1.11 *</td>
</tr>
<tr>
<td>3rd test (D43)</td>
<td>8.53±0.44 *</td>
<td>10.05±0.17 *</td>
</tr>
</tbody>
</table>

Means on the same row with different superscripts are significantly different (P<0.01).

SGPT: Serum glutamic pyruvic Transaminase, SE: Standard Error
NS: Non-significant and S: Significant at 1% level

Fig 1: Normal liver, lung, and heart of rabbit

Fig 2: Normal liver (35gm) in group $T_0$

Fig 3: Hepatomegaly (48gm) $T_1$

Fig 4: Pale liver, heart and lung in group $T_1$

Fig 5: Normal kidney (4gm in group $T_0$)

Fig 6: Enlarged kidney (8gm in group $T_3$)

Fig 7: Congestion in the stomach (internal black arrow) in group $T_3$

Fig 8: Normal stomach (No congestion internal) & intestine (black arrow)
Fig 9: Normal spleen (group T0)

Fig 10: Splenomegaly (group T3)

Fig 11: Microscopic view of pancreas: showing normal histological structure (islets of langerhans cells (black arrow) in group T0 (H and E, Dimension: 947×619)

Fig 12: Microscopic view of pancreas: showing slightly loss of normal histological structure (black arrow) in group T1 (H and E, Dimension: 947×619)

Fig 13: Microscopic view of pancreas: showing decreased beta cell population (black arrow) in the islets of langerhans of pancreas in group T2 (H and E, Dimension: 947×619)

Fig 14: Microscopic view of pancreas: showing necrosis and fibrous tissue proliferation (Black arrow) in interlobular septa and interlobular parenchyma in group T3 (H and E, Dimension: 947×619)

Fig 15: Microscopic view of liver: showing regular pattern of hepatic cord (Black arrow) in group T0 (H and E, Dimension: 947×619)

Fig 16: Microscopic view of liver: showing slightly loss of regular pattern of hepatic cord (Black arrow) in group T1 (H and E, Dimension: 947×619)
Diazinon is an insecticide used in agriculture to control insects and pests on a large variety of fruits and crops. Diazinon exposure is a complicated matter due to efficient absorption of diazinon by inhalation, ingestion and skin penetration and this exposure by the multiple routes can lead to serious additive toxicity. Diazinon poisoned animals showed salivation, lachrymation, diarrhoea and convulsions followed by depression, ataxia and cyanosis as well as death and ensures within a short time.

In the present study with dietary treatment of diazinon body weight of experimental animal significantly (P<0.01) decreased (Table 1) compared to control group which is in agreement with the previous findings of Momena MA, Hattoff DE et al., Rajendra et al. & Nijveldt RJ et al. [28, 12, 31, 30]. Such reduced body weight in the diazinon treated birds could be attributed to the impaired liver function. Liver is the primary organ involved in xenobiotics metabolism and major target organ for the chemical & drug. Xenobiotics toxicity can significantly interfere the gross and microscopic structure as well as enzymatic function which may synergistically results in reduced body weight.

The study shown different gross changes in the different visceral organs, enlargement of kidney, liver (hepatomegaly), spleen (splenomegaly), congestion in stomach (internal) & intestine was recorded in diazinon treated rabbit (Fig.6), (Fig.3), (Fig.10) & (Fig.7). Similar findings were also reported in swine by the study of Earl FL et al. [11].

The microscopic study of liver in current study revealed that group T1 (15mg/kg feed) had slightly loss of regular pattern of hepatic cord (Fig.16) and T2 (30mg/kg feed) showed congestion in some central vein (Fig.17) whereas group T3 (45mg/kg feed) showed highly disarrays of hepatic lobule, necrosis and cirrhosis or fibrous tissue proliferation in liver parenchyma, leucocytic infiltrations and cytoplasmic vaculation of the hepatocytes were also found (Fig.18). This is because that which could be due to the toxic effects of diazinon on liver cells and the vaculation of cytoplasm of hepatocytes may be from extensive lipid infiltration. Gores, G
et al. [18] illustrated that cytoplasmic vacuoles develop due to accumulation of ions and water in cytosol and rapidly pass through leaky membranes of cell organelles. Massive accumulation of fluids in the vacuoles may finally lead to cell lysis & ultimately disarray of structure that is evident in current study.

Microscopic view of kidneys belongs to group T1 showed no remarkable change in kidney tubules but glomerulus seems to be populated with phagocytic/reactive cells (Fig.20). Whereas in group T2 & T3 tubular lining cells were necrosed and thereafter fibrous tissue accumulation, hypertrophy of glomeruli and leucocytic infiltrations was recorded (Fig.21). Microstructure of kidney of control group T0 was normal (Fig.19). The results in this study also agreed with the findings of Afshar, R et al., Hassan. A et al., Saber et al., Ateeq et al. & EI-Shenawy et al. [3, 20, 32, 8, 13] they reported kidney damage with marked tubular dilation, hydropic degeneration in tubular lining epithelium, moderate congestion and hemorrhage in the cortical, lobulated glomeruli of kidneys, of rats. These results indicated that diazinon metabolites caused toxicity in renal system and the immune system made a good role for defending against foreign particles.

Results of the present work indicated that diazinon induced remarkable histopathological alterations in the pancreas of rabbit. Upon microscopic observation, pancreas of treated rabbit with diazinon group T1 (15mg/kg feed) shown loss of normal architecture of parenchyma of pancreas (Fig.12), group T2 (30mg/kg feed) shown degenerative changes in glandular structure & decrease beta cell population (Fig.13) and group T3 (45mg/kg feed) also shown varying degree of degenerative changes, infiltration of inflammatory cells with necrosis and fibrosis (Fig.14). Similarly, the study Gokcimen A et al. [17] reported that intoxicated rat with diazinon resulted in infiltration of inflammatory cells with necrosis of glandular structure (Islets of langerhans) & loss of normal architecture of parenchyma of pancreas. Elias MA Salih [12] reported that the orally administration of ¼ of LD50 of dimethoate and Diazinon for 20 days seriously affected pancreatic beta-cells function.

The following study of Fatima T.A et al. [14] noticed an increase in blood glucose level in diazinon administered rats, but in our experiment there is no significantly increase in blood glucose level (Table 2) such contradiction might be due to the species involvement, route and method of administration of chemical, form of the chemical and duration of exposure of chemical also. The present results also revealed a significant increase in serum creatinine in group T2 and T3 in response to diazinon toxicity (Table 3) that is compliance with the study of EI-Shenawy et al., Ahmed SK & William M et al. [13, 3] in mice. The increase in creatinine recorded in this work might be due to impaired kidney function by the diazinon.

The experimental rabbits exposed to diazinon showed a significant increase in SGPT/AST (P<0.001) concentrations at 43rd days compared to the control group (Table 4). Highest SGPT/AST was found in group T3 (18.90±3.66u/l) followed by group T2 (14.23±1.88u/l) and group T1 (10.05±0.17a/l) compared to control T0 (8.53±0.44u/l). This finding coincides with other previous findings of Ahmed SK, Hattoff DE et al. & Chatterjea MN [3, 21, 10]. Such increased level of ALT is the major diagnostic symptom of liver damage which is evident by gross and microscopic changes in liver such as pale coloration & hepatomegaly in diazinon treated rabbit.

5. Conclusion
Livestock sector in Bangladesh now is under the thread of environmental poison, chemical and insecticide that is use in the crops, fruits etc. The exposure of the diazinon affects the physiology of rabbit which in turn may be the issue of public health through food chain. It was observed from the current study that supplementation of diazinon of rabbit diets at 15, 30 and 45 mg/kg feed produced various deleterious effects on growth performance; gross and microscopic study of different organs as well as biochemical parameters. Decreased body weight in insecticide treated rabbit is due to malabsorption and altered metabolism (hepatotoxicity and nephrotoxicity). The present study revealed various degrees of histological changes that accompanied the biochemical changes in the pancreas, liver and kidney tissues in experimental groups as compared with those of control group. Increased SGPT/ALT in blood of all treated groups resulted from altered permeability of plasma membrane, cellular damage and altered metabolism which was a specific indicator of hepatocellular damage, extrahaepatic obstruction, or both. However, the accumulation rate of insecticide in various organs was not recorded due to lack of technical facilities. Therefore, it is recommended for further study to determine the affinity of insecticide in different organs. Moreover, public health hazards due to insecticide exposure & its preventive strategies should also be undertaken.

6. Acknowledgements
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