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Full Length Research Paper

Immunological and histological effects of exposure to imidacloprid insecticide in male albino rats

Mohamed Mohany[1,2], Gamal Badr[1,3]*, Inas Refaat[2] and Mostafa El-Feki[2]

[1]Zoology Department, College of Science, King Saud University, Saudi Arabia.
[2]Zoology Department, Faculty of Science, Minia University, Egypt.
[3]Zoology Department, Faculty of Science, Assiut University, Egypt.

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Imidacloprid (IC) is a relatively new systemic insecticide related to the tobacco toxin nicotine. The effects of repeated oral administration of IC over 4 weeks on immune response, oxidative stress and hepatotoxicity were assessed. Forty-eight adult male albino rats were divided into two groups of twenty-four animals each. The control group was orally administered distilled water, while the IC-treated group was orally administered 1/100 LD_{50} (0.21 mg/ kg body weight) of IC insecticide daily. We found a significant increase in the total leukocyte count, total immunoglobulins (Igs) especially IgG. In contrast, significant decreases in phagocytic activity, chemokinesis and chemotaxis were observed in the IC-treated group compared to the control group. Histopathologically, the spleen tissues of the IC-treated rats displayed low numbers of lymphocytes, some of which appeared to be pyknotic. However, both fibroblasts and bundles, such as trabeculae, occurred in greater numbers. Similarly, thymus tissues in the IC-treated group showed lymphocytic depletion with pyknotic nuclei. Additionally, significant increases in the serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), alkaline phosphatase (ALP) and malondialdehyde (MDA) were observed in the IC-treated group. Accordingly, in the IC-treated group, heavily congested central vein and blood sinusoids were observed in the liver tissues; pyknotic nuclei were found throughout the hepatic tissue, and leukocyte infiltration was observed. In summary, these results suggest that exposure to 1/100 LD_{50} of IC induces immunotoxicity, oxidative stress, lipid peroxidation and hepatotoxicity.

Key words: Hepatotoxicity, imidacloprid, immunotoxicity, oxidative stress.

INTRODUCTION

The extensive use of insecticides has been criticized in recent years due to their persistence in the environment and their accumulation in the living tissues of organisms. Imidacloprid (IC) is a member of a new group of insecticides called neonicotinoids. It was introduced in response to restrictions on the use of the most commonly used pesticides, chlorpyrifos and diazinon (TDC Environmental, 2003). Since its launch in 1991, imidacloprid has been registered in 120 countries for use on termites and households pests (Liu et al., 2005). Because of its selectivity for insects (Tomizawa and Casida, 2005), IC is thought to be safer than other pesticides (Felsot and Ruppert, 2002), and it now has the highest production of all insecticides worldwide (Ware and Whiteacre, 2004). IC is known to act as a nicotinic acetylcholine receptor (nAChR) agonist (Tomizawa and Casida, 2005).

IC binds to and activates nAChR at its nicotine and acetylcholine binding site, affecting synaptic transmission and leading to disruption of the nervous system (Anatra-Cordone and Durkin, 2005). Although previous studies have found low toxicity to mammals (Anatra-Cordone and Durkin, 2005) and humans, human IC poisoning (David et al., 2007) and two fatal intoxication cases (Proenca et al., 2005) have recently been reported. The potential risks

*Corresponding author. E-mail: badr73@yahoo.com. Tel: 00966509673084. Fax: 0096614678514.

from IC have therefore gained significant attention due to its worldwide use and persistence in crops. Laboratory studies of IC have shown genotoxicity in both rats (Karabay and Oguz, 2005) and cultured human lymphocytes (Demsia et al., 2007). Moreover, neurobehavioral deficits were found in rats exposed to IC in the uterus (Abou-Donia et al., 2008).

Although there is a large body of literature addressing immune responses during insecticide exposure, the immunotoxicity of IC is poorly understood. Due to its importance in assessing drugs and non-drug chemicals, immunotoxicity testing is required by many regulatory agencies (Holsapple, 2003). There have been few studies of IC's immune system effects using hemagglutinating antibody titer (HAT), delayed type of hypersensitivity (DTH) response, leukocytic migration inhibition (LMI) against sheep RBCs and the phagocytic index. Progressive and proportional decreases in HAT and DTH response were observed in IC treated rats. The phagocytic index and leukocytic migration were also found to be reduced, suggesting immunotoxic effects (Gatne, 2006).

Recently published studies have indicated that pesticide toxicity may be associated with the enhanced production of reactive oxygen species (ROS), which could explain the multiple types of toxic responses observed. The production of ROS is caused by a mechanism in which xenobiotics, toxicants and pathological conditions may produce oxidative stress and induce damage to the liver, kidney and brain tissue (Yu et al., 2008; El-Gendy et al., 2010). Increased free radical generation and/or decreased antioxidant levels in target cells leads to oxidative stress, which damages macromolecules such as nucleic acids, lipids and proteins, alters the function of the cell and leads to cell death (Stephan et al., 1997). Damage to membrane lipids, proteins and DNA is considered the end biomarker of the oxidative stress inducing effects of pesticides (Tuzmen et al., 2008). The toxic effects of pesticides on humans, particularly those from free radical production, can be confirmed by the direct measurement of malondialdehyde (MDA), which is the end product of lipid peroxidation (Muniz et al., 2007).

The liver is the principal target of IC toxicity, as demonstrated by the following: Hepatic necrosis or hypertrophy; elevated serum transaminase, alkaline phosphatase and/or glutamate dehydrogenase activities; and alterations of other clinical chemistry parameters, such as uric acid, glucose, cholesterol, total protein and albumin after oral ingestion (Kammon et al., 2010).

Although pesticides are known to impair immune responses in addition to inducing oxidative stress in numerous animal models, very few data are available concerning IC immunotoxicity. Therefore, this study aimed to increase our understanding of the immunotoxic effects of IC exposure and the role of oxidative stress in the pathogenesis of these effects.

MATERIALS AND METHODS

Experimental animals

Forty-eight adult male albino rats, *Rattus norvegicus* weighing 150 to 170 g were obtained from the General Organization of Serum and Vaccine (GOSV), Helwan farm, Egypt. All animal procedures were performed in accordance with the guidelines for the care and use of experimental animals of the Committee for Purpose of Supervision of Experiments on Animals (CPCSEA) and the National Institutes of Health (NIH). The study protocol was approved by the Animal Ethics Committee of the Zoology Department, College of Science, Minia University and according to Helsinki principles. The animals were allowed to acclimatize in metal cages inside a well-ventilated room for 2 weeks prior to the experiment. They were maintained under standard laboratory conditions (23°C, relative humidity 60 to 70% and a 12-h light/dark cycle) and were fed a diet of standard commercial pellets and water.

Insecticide and administration

Imidacloprid (IC) is produced by Bayer Company under the trade name Confidor. The commercial Confidor 20% EC formulation was obtained from Bayer Scientific Office in Cairo, Egypt. IC was administered orally with a blunt ended syringe needle; 1 ml of Confidor 20% EC was dissolved in 99 ml of distilled water and administered at a dose of 1/100 LD_{50}, 0.21 mg/kg in a solution that was freshly prepared within 5 min of administration.

Experimental design

Various concentrations of imidacloprid (IC) were dissolved in distilled water and administered to rats orally with a single dose. Rats serving as controls received distilled water only. After 24 h, the LD_{50} toxicity value was calculated. The effects of IC on immune response, oxidative stress, and the liver enzymes of male rats were studied by dividing the animals into two groups of 24 animals each. The control group was orally administered distilled water, which is the solvent used for the IC formulations, daily for four weeks. The IC-treated group was administered daily oral treatments of 1/100 LD_{50} (0.21 mg/kg) of IC insecticide for four weeks.

Collection of blood and tissue samples

After each week, six rats each from the control and treatment groups were killed and two blood samples were immediately collected. The first sample was collected in a heparinized tube (2.25 µl heparin/5 ml blood) for chemotaxis, chemokinesis, phagocytosis and white blood cell count assays. The second sample was collected in a non–heparinized tube and centrifuged for 10 min at 3000 rpm to separate the serum, which was then stored at -80ºC to measure immunoglobulin content and other biochemical parameters. After dissection of the animals, organs including the liver, spleen, and thymus were removed, wiped with filter paper and fixed in Bouin's fixative for histopathological examination.

Total leukocyte count and phagocytosis

White blood cells were counted as previously described (Schalm et al., 1975). The phagocytic activity of macrophages and other phagocytic cells in whole blood was examined using phase contrast microscopy; the whole blood was stored in cold 0.9% saline solution, and one drop of each sample was examined with phase contrast microscopy in a drop of paraffin oil. Phagocytosis ratios were calculated and notable phagocytic states were photographed

(Abu El-Maged, 1991).

Micropore filter assay for chemokinesis

In an attempt to more precisely quantify the chemotactic response, a modified Borden's chamber assay was used (Boyden, 1962). In a Borden's chamber assay, cells migrate through a micropore filter from a starting compartment into another containing a chemoattractant.

For the method employed here (Gearing and Rimmer, 1985; Abu El-Maged, 1991; El–Feki, 1994), the two compartments were formed by the wells of two microtitration plates on top of each other, with cellulose acetate micropore filters (5 µm millipore 67/20 Molsheim, France) partitioning the wells. The lower test wells were filled with chemoattractant (SRBCs) or, as a control, saline (0.9% NaCl). Five-micrometer micropore filters were then placed over all lower wells.

The upper plate was inverted and placed over the lower plate, with petroleum jelly around the perimeter joining the plates. Blood (0.3 ml) was introduced into each chamber through a hole made in the base of each well. The whole plate was then covered with aluminum foil to reduce evaporation and placed in humid incubator at 37°C for 16 to 18 h. Following incubation, the fluid was removed from the upper wells with a syringe and needle and replaced with 0.3 ml of methanol to fix the cells. After 20 min, the methanol was removed and the two plates were separated. The filter papers were then fixed in 10% formalin for 20 min, stained in Delafield's hematoxylin for 5 min, washed with distilled water and mounted in Canada balsam on a microscopic glass slide with the bottom surface facing upward. Two counts were made on each of 5 filters. The chemokinetic effect obtained was expressed using a chemokinetic index calculated as:

Chemokinetic index = Mean number of cells per h.p. field with stimuli divided by the mean number of cells per h.p. field with control stimulus

Agarose gel chemotaxis movement assay

A chemotaxis assay was used to study the chemotactic response of leukocytes in response to imidacloprid. A previously described method (Nelson et al., 1975, Comer et al., 2005), in which cells migrate from a well cut through an agarose gel into a well containing a chemotactic stimulus, was employed.

The assay was performed in sterile 60 × 15 Petri dishes. Agarose gel was prepared in 100 ml volumes by dissolving 0.8 g of agarose (BDH) in 38 ml of 0.9% saline solution in a boiling water bath for 20 min. One milliter of 1% fetal calf serum (FCS; flow) and 1 ml of combined penicillin and streptomycin (flow) were added to the gel as it cooled. The FCS was sterilized by filtering through a 0.22 mm disposable millex filter unit.

The mixture was delivered to the petri dishes in 10 ml aliquots using a pre-warmed sterile pipette. The gel was then allowed to set on a level table at room temperature and then stored at 4°C until use. For each assay, two groups of three 2.5 mm diameter wells separated by 2.5 mm were made in the gel.

Three Petri dishes were used for each test, corresponding to six replicates for each. The central wells received 10 µl of blood. The wells on one side were used as controls and received 0.9% saline solution. The plates were covered and incubated for 16 to 18 h at 38 to 42°C and 95% humidity. The migrated cells were fixed in methanol for 30 min. The plates were coded and read independently by two separate observers. The observed chemotactic effect was expressed using a chemotactic index calculated similarly to the chemokinetic index.

Turbidity test for estimation of total immunoglobulin level (Total Ig)

Two widely used procedures for measuring immunoglobulin levels are single radial immunodiffusion (RID) (Meguire et al., 1976) and zinc sulfate (ZnSO4) turbidity. The latter is a "salting out" procedure that depends on the biochemical properties of immunoglobulin in relation to the characteristics of $ZnSO_4$. In this study, this technique was performed as previously described (Mcewan et al., 1970; Pfeiffer et al., 1977).

Determination of serum IgG and IgM

The procedure to determine serum IgG and IgM consists of an immunoprecipitation in agarose between an antigen and its homologous antibody and was performed as previously described (Berne, 1974). The procedure is performed by incorporating an immune reactant (usually an antibody) uniformly throughout a layer of agarose gel and then introducing the other reactants (usually antigens) into wells carved in the gel. Antigen diffuses radially out of the well into the surrounding gel-antibody mixture, and a visible ring of precipitate forms where the antigen and antibody react. There is a quantitative relationship between ring diameter and antigen concentration. While the precipitate is expanding, the ratio between ring diameter and antigen concentration logarithm is approximately linear. This relationship is useful when rapid estimations are required.

Liver enzyme assays and lipid peroxide (Malondialdehyde)

Aspartate and alanine transaminases were assayed with a colorimetric method (Reitman and Frankel, 1957) using a Roche Diagnostic Reflotron. Serum alkaline phosphatase activity was determined as described previously (Belfield and Goldberg, 1971) using reagent kits purchased from a biodiagonstic chemical company (Egypt). Thiobarbituric acid reactive substances (TBARS), the final products of lipid peroxidation, were assayed spectrophotometrically as described previously (Satoh, 1978) and measured as malondialdehyde (MDA).

Histopathology

The liver, spleen and thymus were preserved in 10% formalin immediately following their removal from the animal.

Tissue processing

The liver, spleen and thymus tissues were placed in 10% formalin (diluted to 10% with normal saline) for 1 h to reverse the shrinkage that had been caused by the high concentration of formalin. The tissues were dehydrated in an ascending grade of isopropyl alcohol by immersion in 80% isopropanol overnight and in 100% isopropyl alcohol for 1 h. The dehydrated tissues were cleared in two changes of xylene for 1 h each. The wax-impregnated tissues were embedded in paraffin blocks using wax of the same grade. The paraffin blocks were mounted and cut with a rotary microtome at a 3-micron thickness. The sections were floated in a tissue floatation bath at 40°C and placed on glass slides that were smeared with equal parts of egg albumin and glycerol. The sections were then melted in an incubator at 60°C for 5 min and allowed to cool.

Tissue staining

The sections were deparaffinized by immersion in xylene for 10 min

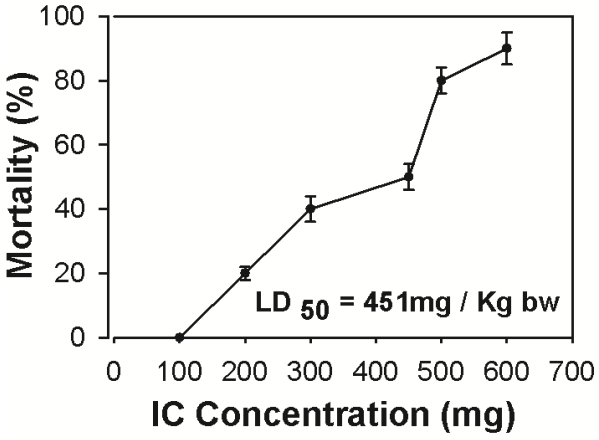

Figure 1. Determination of the LD_{50} of IC in male rats as 451 mg/kg. Results are expressed as means ± SEM. * P<0.05.

in a horizontal staining jar. The deparaffinized sections were washed in 100% isopropyl alcohol and stained in Ehrlich's hematoxylin for 8 min in a horizontal staining jar. After staining in the hematoxylin, the sections were washed in tap water and dipped in acid alcohol to remove the excess stain (8.3% HCl in 70% alcohol). The sections were then placed in running tap water for 10 min to promote bluing (slow alkalization). The sections were counterstained in 1% aqueous eosin (1 g in 100 ml tap water) for 1 min, and the excess stain was washed in tap water before the sections were dried. The complete dehydration of the stained sections was achieved by placing the sections in an incubator at 60°C for 5 min. When the sections were cooled, they were mounted in DPX mount, which has the optical index of glass (the sections were wetted in xylene and inverted onto the mount and then placed on the cover slip). The architecture was observed using a low-power objective under a microscope.

Statistical analysis

Data are expressed as means ± SE. Statistical analysis was performed using one-way ANOVA followed by a post-hoc test for multiple comparisons within SPSS 10.0 for Windows. P-values less than 0.05 were considered statistically significant.

RESULTS

Toxicity study

Oral administration of different concentrations of IC gave an LD_{50} value of 451 mg/kg for male rats (Figure 1).

Short-term oral administration of IC inhibits innate immunity

We monitored changes in immunological parameters related to innate immunity in both animal groups. We observed a significant increase in total leukocyte count compared with the control group after oral administration of 1/100 LD_{50} IC (Figure 2A). The treated animals' immune response kinetics was determined by measuring phagocytosis, chemokinesis, and leukocyte chemotaxis in whole blood. IC administration induced a significant decrease ($P<0.05$) in leukocyte phagocytic activity, with the lowest value (19.3± 2.2) recorded in the 4th week (Figure 2B). The chemokinetic index and leukocyte chemotaxis also significantly decreased ($P<0.05$) compared with control rats, and both reached their minimum in the 4th week (Figure 2C and D).

IC toxicity increases serum immunoglobulin levels

We next investigated changes in serum immunoglobulins due to short-term oral IC administration. IC treatment induced a significant increase in total Ig and IgG levels ($P<0.05$) compared with the control group, with the highest values observed in the 4th week (Figure 3A and B). However, no post-IC administration changes in serum IgM were observed (Figure 3C).

Oral IC administration alters the histological structure of both the spleen and the thymus

We investigated whether short-term oral administration of IC affects the histological structure of the spleen and thymus. The white pulp spleen sections of animals treated with 1/100 LD_{50} of IC insecticide for 4 weeks were greater in size and had low lymphocyte densities, more fibroblasts, red pulp congested with red blood cells, more bundles such as trabeculae, and some pyknotic lymphocytes (Figure 4B). In comparison, the normal spleens in the control group displayed splenic parenchyma composed of white and red pulp surrounded by a capsule of dense connective tissue, from which emanated trabeculae dividing the splenic parenchyma into incomplete compartments (Figure 4A). When compared to the thymi of control rats (Figure 4C), the thymi of animals treated with 1/100 LD_{50} of IC for 4 weeks showed lymphocytic depletion, lymphocyte invasion, fibroblasts, occasional eosinophilic cells, pyknotic nuclei and focal areas of macrophage activity (Figure 4D).

IC toxicity increases liver enzymes and lipid peroxidation

We also monitored hepatic dysfunction due to IC toxicity by measuring aspartate aminotransferase (AST),.alanine aminotransferase (ALT) and alkaline phosphatase (ALP) liver enzymes. As shown in Figure 5A and B, AST and ALT were found to be significantly increased post-IC administration compared with control rats ($P<0.05$), with the highest values (62.3±2.3, 85.7±1.7) recorded in the 3rd and 4th weeks, respectively. ALP exhibited a

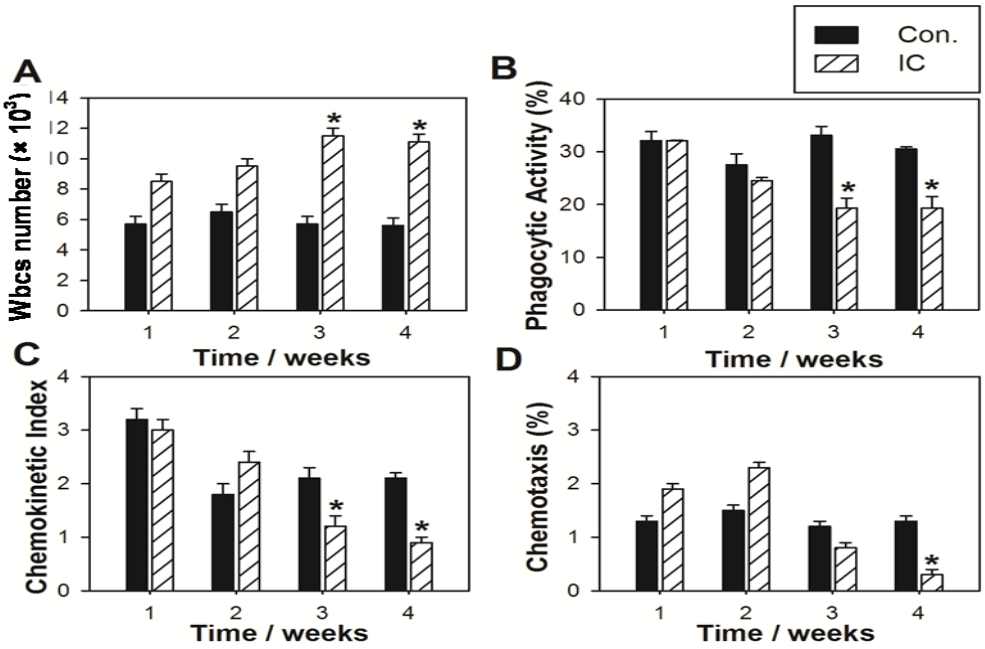

Figure 2. Changes in innate immunity due to short-term oral administration of 1/100 LD_{50} of IC over 4 weeks. Effects of IC oral administration of 1/100 LD_{50} on total leukocyte count ($N \times 10^3$), (A). Phagocytic activity (%) of male rats orally treated with IC (1/100 LD_{50}), (B). Chemokinetic leukocyte index of male rats orally administered IC (1/100 LD_{50}), (C). Leukocyte chemotaxis of male rats orally treated with IC (1/100 LD_{50}), (D). Values are given as means ± SEM. *P<0.05.

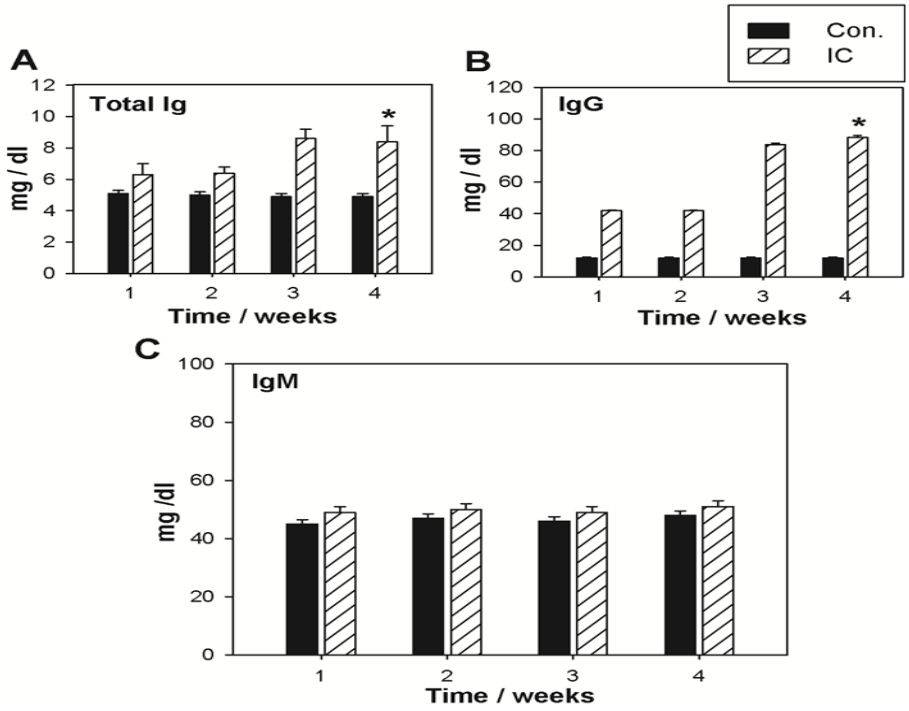

Figure 3. Changes in immunoglobulin levels due to short-term oral administration of 1/100 LD_{50} of IC over 4 weeks. Changes in serum total Ig (mg/dl) after 4 weeks of oral IC administration (1/100 LD_{50}) (A). Levels of serum IgG (mg/dl) in male rats orally treated with IC (1/100 LD_{50}) (B). Levels of serum IgM (mg/dl) in male rats orally treated with IC (1/100 LD_{50}) (C). Values are expressed as means ± SEM. *P<0.05.

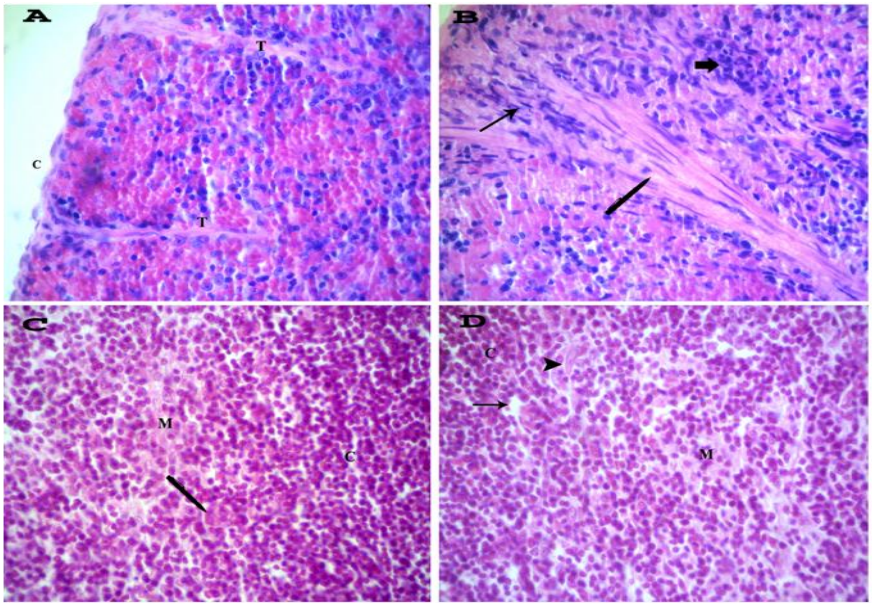

Figure 4. Oral administration of IC induces marked alterations in the histology of both the spleen and the thymus. Sections of control rat spleens showing outer capsules (C) and extending trabeculae (T) (H&E. ×500) (A). Spleen sections from rats treated with 1/100 LD_{50} of IC insecticide for 4 weeks showing fibroblasts (thin arrows), large bundles of fibers (pen arrows), and small nests of pyknotic nuclei (thick arrows) (H&E. ×500) (B). Section of the thymus of a control rat showing the cortex (C) and medulla (M). Note Hassle's corpuscle (pen arrow). (H&E. x500) (C). Section of the thymus of a rat treated with 1/100 LD_{50} of IC for 4 weeks showing a cortex (*C*) and medulla (*M*) with lymphocytic depletion, invasion of fibroblasts (head arrow) and focal areas of macrophage activity (thin arrow). (H&E. ×500), (D).

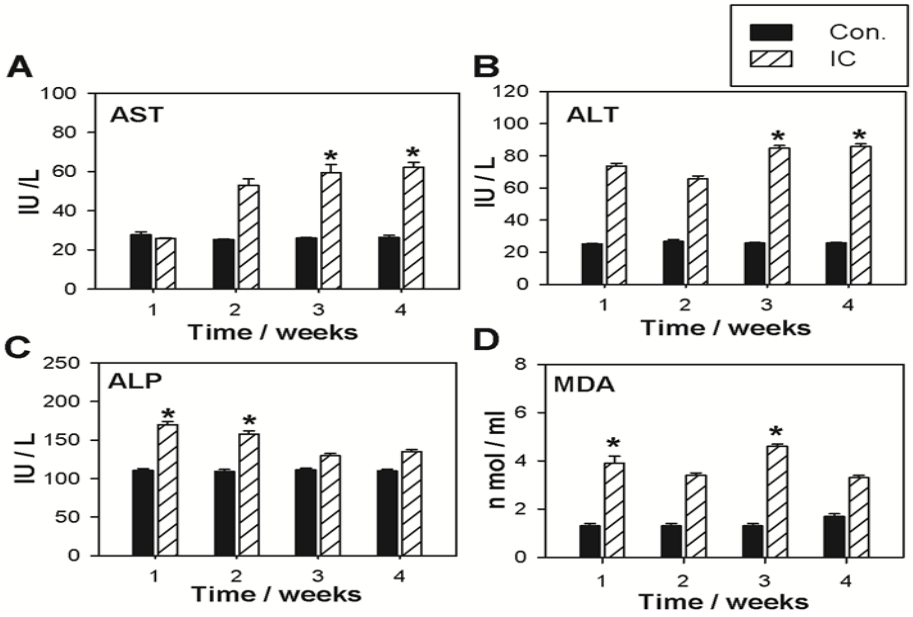

Figure 5. Changes in serum hepatic enzyme and MDA levels due to short term oral administration of 1/100 LD_{50} of IC for 4 weeks. Effects of oral administration of 1/100 LD_{50} of IC on serum AST (IU/L) (A). Changes in serum ALT (IU/L) after 4 weeks of oral IC administration (1/100 LD_{50}) (B). Levels of serum ALP (IU/L) in male rats orally treated with IC (1/100 LD_{50}) (C). Effects of oral administration of 1/100 LD_{50} of IC on serum MDA (n mol / ml) (D). Values are given as means ± SEM, * = $P<0.05$.

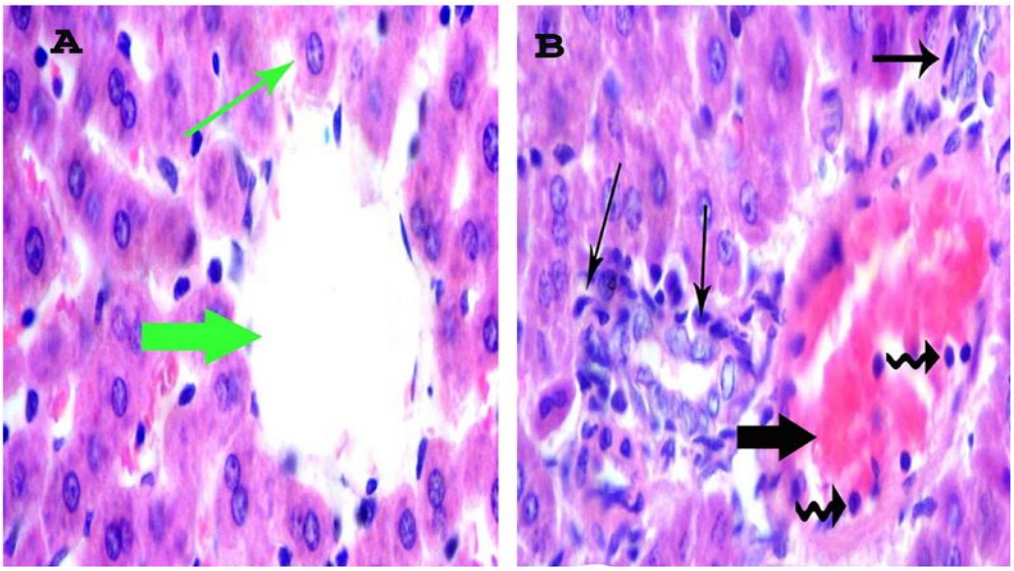

Figure 6. Oral administration of IC induces marked alterations in liver histology. Liver of a control rat showing normal hepatocytes (thin arrow) and central vein (thick arrow) (H&E. ×500) (A). Liver section of a rat that was administered 1/100 LD_{50} of IC for 4 weeks showing hepatocytes with homogenous cytoplasm, a central vein congested with red blood cells (thick arrow), infiltration of leukocytes (zigzag arrow) and a fibroblast around the bile duct and central vein (thin arrow) (H&E. ×500) (B).

significant increase ($P<0.05$) post-IC administration compared with the control group, with the highest values observed in the 1st and 2nd weeks (Figure 5C) and malondialdehyde (MDA) is a marker of oxidative lipid damage and a major oxidative product of peroxidized polyunsaturated fatty acids (Zhang et al., 2004). MDA levels significantly increased in the IC-treated group compared to the control group, with the highest levels recorded in the 1st and 3rd weeks (Figure 5 D).

Short-term oral administration of IC alters the histological structure of the liver

When compared with those of control rats (Figure 6A), liver sections of animals treated with 1/100 LD_{50} of IC insecticide for 4 weeks showed heavily congested central vein and blood sinusoids, widely distributed pyknotic nuclei, and leukocyte infiltration (Figure 6B).

DISCUSSION

The kinetics of the immune responses of the treated animals was measured via their innate and humoral responses. The first and often most important response to infectious agents is non-specific immunity. This includes the soluble and cellular factors of acute inflamamation, various serum proteins such as complement proteins and circulating and tissue phagocytes (Adedeji et al., 2009). The primary function of white blood cells is defense against foreign bodies, which is achieved by leukocytosis and antibody production**.** The total leukocyte count was higher after treatment with IC insecticide. Our results agree with those found by Ammar et al. (2003), who reported that 0.1 and 0.25 LD_{50} of IC insecticide induced significant increases in the total leukocyte counts of male albino rats. This increase may be related to an increase in lymphocyte number (data not shown). Phagocytosis is the principle mechanism used by mammals to destroy extracellular pathogens and several viral and fungal organisms (Kantari et al., 2008). In this study, treatment with IC insecticide was observed to decrease phagocytic activity. This reduction may be due to suppression of the antibody response (El-Gohary et al., 2005), the action of pesticides on hematopoietic organs, the prevention of macrophage arming factor uptake, or increased migration inhibition factor (MIF) activity induced by IC, which suppresses the mobility of macrophages and neutrophils and their ability to reach inflammatory sites.

In response to the presence of chemical substances in the environment, immunocompetent cells (that is, leukocytes) are often seen to migrate to and become localized at the sites of inflammation. When leukocyte migration is directional along an increasing concentration gradient of an attracting substance, it is termed “chemotaxis.” If the response to the substance is an increase in the speed or frequency of migration, it is termed “chemokinesis” (Obenauf and Smith, 1985). In the present study**,** both chemotactic and chemokinetic activities significantly decreased after IC administration, which may be related to an inhibitory effect of IC

described by Michel et al. (2000).

Total immunoglobulin levels significantly increased after IC administration. As observed by Neishabouri et al. (2004), this increase may be due to lymphocyte activation. Furthermore, the increase in total Ig may be related to the formation of anti-imidacloprid antibodies as detected by the previous precipitation technique. IC toxicity was observed to induce a significant increase in IgG without any significant changes in IgM levels. Similar observations have been made by Klucinski et al. (1996), who found that workers exposed to multiple insecticides had increased serum IgM, IgG and IgA concentrations; these increases may be attributable to inflammation induced by these insecticides. IgGs are antibodies produced in response to Th2 activation (Ormstad et al., 2003).

Our data show that the spleens of rats treated with 1/100 LD_{50} 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 et al. (2008), 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 (Hsu and Mountz, 2003).

The thymus is the site of T cell differentiation and maturation. The thymic tissue of rats treated with 1/100 LD_{50} of imidacloprid for 4 weeks showed many changes when compared with that of normal rats. These changes included lymphocytic depletion; invasion by lymphocytes, fibroblasts, and occasional eosinophilic cells; pyknotic nuclei; and focal areas of macrophage activity. This histological architecture has been described as an indication of thymic involution (atrophy). Similar observations have been made by Lukowicz-Ratajczak and Krechniak (1992), who reported that deltamethrin insecticide, a cyano-pyrethroid, affects both cellular and humoral immune responses in mice.

A marked increase in serum AST, ALT and ALP was observed after the administration of 1/100 LD_{50} of IC. These results are similar to those of Balani et al. (2011) in a study of male White Leghorn (WLH) chicks treated with different concentrations of IC. This increase in level of specific enzymes correlates well with the gross and histopathological changes in livers we observed. MDA levels significantly increased in the IC-treated group compared to the control group. Kapoor et al. (2010) investigated the effects of IC on antioxidant enzymes and lipid peroxidation and made similar observations. They suggested that oxidative metabolites and/or free radicals are produced during IC metabolism. Histopathological examination of liver tissues exposed to IC showed heavily congested central vein and blood sinusoids, widespread pyknotic nuclei in the hepatic tissue, and leukocyte infiltration. These results are in accordance with histopathological lesions observed in livers of Japanese quail exposed to IC for 6 weeks (Omiama, 2004) and in layer chickens exposed to 139 mg/kg of IC (Kammon et al., 2010).

In conclusion, exposure to 1/100 LD_{50} of IC produced immunotoxicity, oxidative stress, lipid peroxidation and hepatotoxicity.

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