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MODULATORY EFFECTS OF THYMOQUINONE ON THE HISTOLOGICAL CHANGES INDUCED BY IMIDACLOPRID IN ALBINO RATS

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ABSTRACT

The possible modulatory effects of thymoquinone (TQ) against pathological changes induced by imidacloprid (IC) were examined using male albino rats. One hundred and forty four adult male albino rats *(Rattus norvegicus*) were allocated into six groups, one group is normal, two are control groups and the other are treated groups. IC was given orally at a dose of 1/100 LD_{50} / day for 4 weeks, without TQ, before TQ, or with TQ (given as a single i.p. injection weekly at a dose of 1mg/ kg.b.wt). Histopathological studies of liver showed dense interstitial hemorrhage, pyknotic nuclei, and infiltration with leucocytes. Also, kidney sections of animals treated with IC insecticide showed clear proliferation, interstitial hemorrhage, and pyknotic nuclei in glomerular tissue. Furthermore, marked dilatation in renal tubules and urinary spaces were observed. Medullary tubules of the kidney showed interlobular hemorrhage. Partial recovery of histological changes was observed after TQ supplementation. It is concluded that TQ might alleviate histological alteration in the liver and kidney which are induced by IC.

Key words: Thymoquinone (TQ), Imidacloprid (IC), Histological changes, Albino rat.

INTRODUCTION

The extensive use of insecticides in general has given rise to criticism in recent years, due to their persistence in the environment and their accumulation in the living of organism tissues (Saleh *et al.*, 1998). Due to increasing insecticide application, it has become necessary to assess their environmental hazards to human health. IC (Figure 1), is an agonist to the nicotinic acetylcholine receptors and as such it is highly effective against many sucking insects (Elbert *et al.*, 1998).

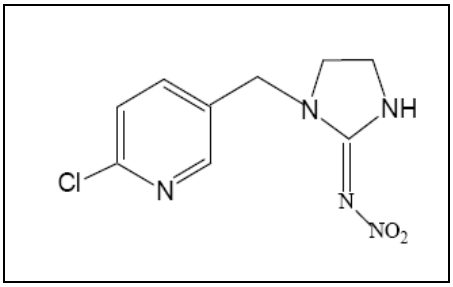

Figure (1): Chemical structure of imidacloprid insecticide.

This chemical works by interfering with the transmission of stimuli in the insect nervous system. It causes a blockage in a type of neuronal pathway that is more abundant in insects than in warm-blooded animals. This blockage leads to the accumulation of acetylcholine, resulting in the paralysis, and eventually death of insects (Kidd and James, 1991). Histological techniques are widely used as a potential toxicity marker for different environmental pollutants including insecticides (Bhuiyan *et al.*, 2001).

Thymoquinon (TQ), a pharmacologically bioactive quinone of *Nigella sativa* (Black cumin), has shown to be anti-cancer, analgesic and anti-inflammatory (Abdel-Fattah *et al.*, 2000). Moreover, studies revealed its protective effect against hepatotoxicity in mice exposed to carbon tetrachloride (Daba and Abdel-Rahman, 1998), cardiotoxicity in mice treated with doxorubicin (Al- Shabanah *et al.*, 1998) and nephrotoxicity induced by cisplatin, isosfamide, and gentamycin (Badary *et al.*, 1999). Its ability to prevent the membrane lipid peroxidation in hepatocytes induced by carbon tetrachloride or *tert*- butyl hydroperoxide *in vitro* (Daba and Abdel-Rahman, 1998) and *in vivo* (Mansour *et al.*, 2001) and on brain (Hosseinzadeh and Montahaei, 2007) indicated its potent antioxidant activity and suggest a prophylactic role in preventing the consequences of the oxidative injury. In a study on

rat treated with doxorubicin, TQ was capable of suppressing severe nephrotic syndrome associated with an increase in the urinary excretion of protein, total glycerides, total cholesterol, and lipid peroxides (Badary *et al.*, 2000).

The aim of the present work was to clarify the modulatory role of TQ on the toxic effects of IC on the liver and kidney of albino rats (*Rattus norvegicus*), using histological techniques.

MATERIALS AND METHODS

A- Experimental animals:

One hundred and forty four adult male albino rats *Rattus norvegicus*, weighing from 150 – 170g were obtained from the General Organization of Serum and Vaccine (GOSV), Helwan farm. The animals were kept in the animal house, faculty of science, Minia University. All animals were housed in metal cages and provided with rodent dry pellet and water *ad libitum* under good hygienic laboratory conditions for two weeks before the beginning of the experiments.

B- Chemicals:

IC (Bayer Company under the trade name confidor 20% EC) was orally administered by using a blunt ended syringe needle in total volume of 1 ml of insecticide solution. The sample of insecticide formulation obtained from Plant Protection Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki and Giza. 1 ml of confidor 20 % EC was dissolved in 99 ml of distilled water and the used dose (1/100 LD_{50}, 0.21mg / Kg B.w.). TQ was purchased from Sigma Chemical Company (St Louis, MO, USA), and reconstituted in TWEEN 20% at a concentration of 4mg/ml. This stock was stored at 4°C in 15ml centrifuge tubes wrapped in aluminum foil to avoid dimer formation.

C- Experimental design:

Animals were divided into six groups, three of them are control groups and the other three are treated groups (twenty four animals in each). The first group (normal) was free injected. The second group (control $_{I}$) was daily orally intubated with 1 ml / kg b.wt. of distilled water which is the solvent of IC insecticide. The third group (control $_{II}$) was injected intraperitonealy once every week with 0.1ml / kg b.wt. of 20% TWEEN the solvent of TQ for two months. The fourth group (treated $_{I}$) was daily orally intubated with 1/100 LD_{50} (0.21mg / kg b.wt.) of IC insecticide for one month, then it was injected intraperitonealy once every week for another one month with 1mg / kg b.wt. of TQ solution. The fifth group (treated$_{II}$) was injected intraperitonealy once every week with 1mg/kg.b.wt.of TQ for one month, and then the next month the animals received daily the dose of IC insecticide. The 6th group (treated $_{III}$) received TQ and IC simultaneously for two month. Both control and treated groups were sacrificed every 4 weeks. After animal dissection the liver and kidney were excised , wiped with filter paper and fixed in 10% neutral buffered formalin and in Bouin's fixative for histopathological examinations.

RESULTS

Liver:

a- Normal group:

The basic structure of normal liver sections stained with Haematoxylin and Eosin showed numerous hepatic lobules. Each of which consists of cords of regularly arranged hepatocytes enclosing the sinusoidal network. The central vein is located in the middle of the lobule. The hepatocytes are polygonal in shape with granulated eosinophilic cytoplasm and centrally located nuclei with one or two nucleoli and delicate strands of chromatin. Also, kupffer cells appeared between the hepatocytes as spindle-shaped cells (Figure 2).

B- Control groups:

The histological changes of the liver sections of control groups showed no remarkable changes when compared with normal group.

c- Treated groups:

Liver sections of animals treated with 1/100 LD_{50} of IC insecticide for 4 weeks showed heavily congested central vein and blood sinusoids, some pyknotic nuclei were noticed allover the hepatic cells, and infiltration of leucocytes (Figure 3). however TQ supplementation post-IC treatment improved the pathological changes which noticed in the IC treated rats except the congestion of blood sinusoids with blood and appearance of some kupffer cells (Figure 4). Concerning the pathological changes of rats treated with IC post TQ period, it was noticed that liver sections showed vacuolar degeneration and individual cell necrosis. In addition, the hepatic sinusoids and blood vessels were focally congested (Figure 5) i.e. the pretreatment of TQ failed to protect liver organ against IC chronic toxicity. Regarding the liver of rats treated with TQ and IC together, it was noticed a recovery of injury except dilation of central vein and congestion of hepatic sinusoids with blood (Figure 6).

Kidney:

a- Normal group:

The kidney consists of an outer cortex and an inner medulla. The outer cortex of normal kidney

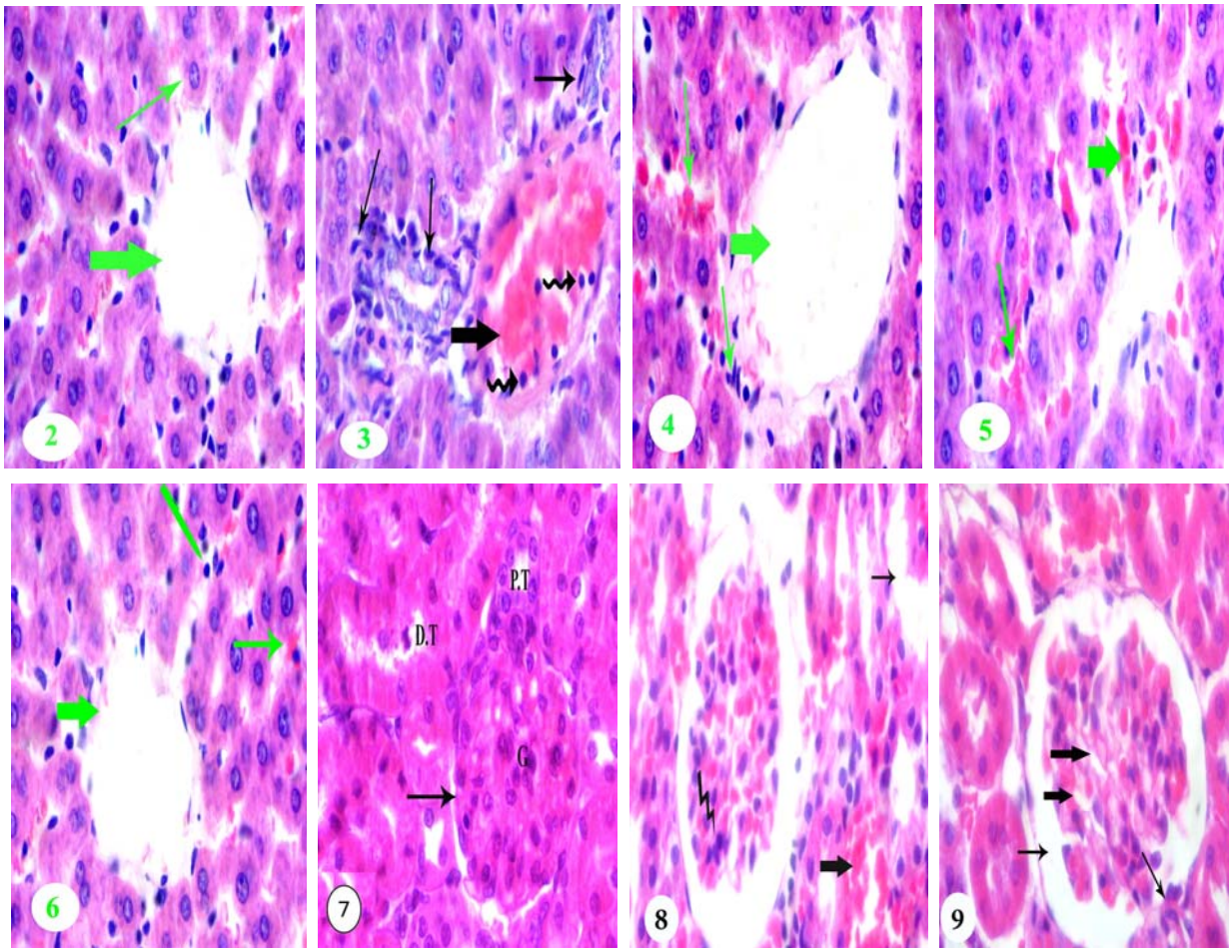

Plate (A):

Fig. (2): Section of liver of normal rat, showing the normal hepatocytes (thin arrows) and central vein (thick arrow).

Fig. (3): Section of liver of treated rat given 1/100 LD_{50} of IC for 4 weeks, showing hepatocytes with homogenous cytoplasm, congested central vein (thick arrow) with red blood cells, infiltration of leucocytes (zigzag arrows) and fibroblast around bile duct and central vein (thin arrows).

Fig. (4): Section of liver of treated rat given TQ (1mg/kg.b.wt.) post 1C treatment for 4 weeks, showing nearly normal central vein (thick arrow). Little number of fibroblosts and red blood cells appear in (he liver sinusoids (thin arrows).

Fig. (5): Section of liver of treated rat given IC (1/100 LD_{50}) post- TQ supplementation for 4 weeks, showing congested central vein (thick arrow) and congested hepatic sinusoids (thin arrow).

Fig. (6): Section of liver of treated rat given TQ with IC for 4 weeks, decongestion of central vein (thick arrow) with pyknotic nuclei (pen arrow) and the congestion of liver sinusoids with blood cells (thin arrow).

Fig. (7): Cross section in the cortex of normal kidney, illustrating the normal appearance of the proximal convoluted tubules (P.T), distal convoluted tubules (D.T), Bowman's capsule (thin arrow) and glomerulus (G).

Fig. (8): Cross section in the cortex of kidney of a rat treated with 1/100 LD_{50} of IC insecticide for 4 weeks, showing massive interstitial hemorrhage (thick arrow), dilatation of renal tubules (thin arrow), mitotic division (lightening arrow) in the glumerular tuft.

Fig. (9). Cross section in the cortex of kidney of a rat treated with TQ (I mg/kg b. wt.) post-IC treatment for 4 weeks, showing renal tubules with homogenous cytoplasm, high vacuolation and hemorrhage in glomcrulus (thick arrows).

Note dilatation in urinary space and aggregation of pyknotic cells (thin arrow). (H&E, x 500).

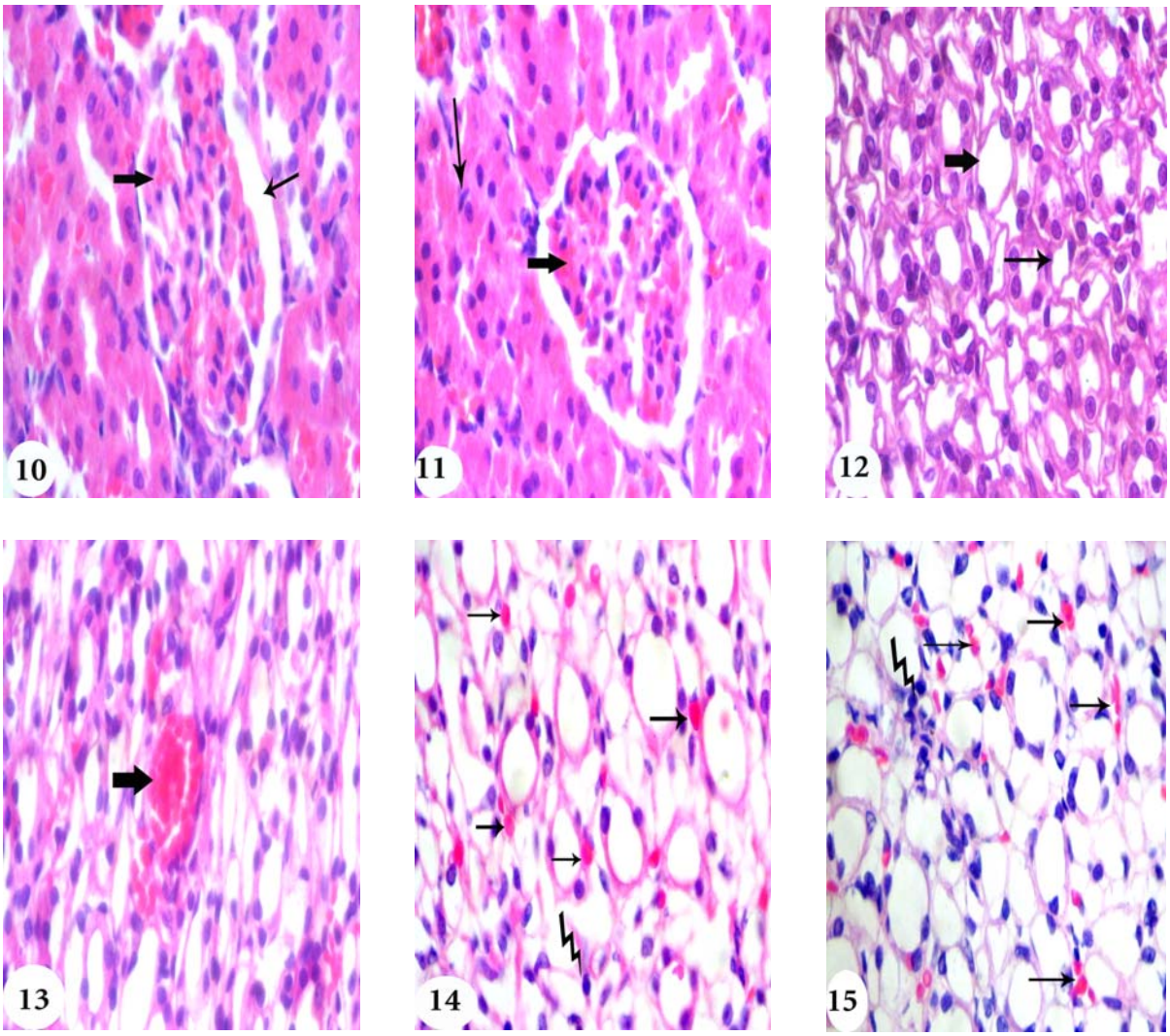

Plate (II):

Fig. (10): Cross section in the cortex of kidney of a rat treated with 1/100 LD50 of IC post-TQ supplementation for 4 weeks, showing recovery features as slight hemorrhage in renal tubules and glomerulus (thick arrows). Not the urinary space (thin arrow).

Hy. (11): Cross section in the cortex of kidney of a rat treated with TQ and IC lor 4 weeks, showing modulated homogenous cytoplasm, nearly normal glomerulus but with little hemorrhage (thick arrow), and few fibroblasts (thin arrow),

Kip. (12): Cross section in the medulla of a normal kidney, illustrating the normal appearance of the medulla which consists of the ascending c.t (thick arrow), descending c.t (thin arrow) loop of Henel.

Fig. (13): Cross section in the medulla of the kidney of a rat treated with 1/100 LD_{50} of IC for 4 weeks, showing severe interlobular hemorrhage (thick arrow),

Fig. (14): Cross section in the medulla of the kidney of a rat treated with 1 mg/kg b.wt. of TQ post- IC administration for 4 weeks, showing interlobular hemorrhage (thin arrow) and pyknotic nuclei (lightening arrow).

Fig. (15): Cross section in the medulla of the kidney of a rat treated with IC post-TQ for 4 weeks, showing dilatation in lumen of renal tubules, pyknotic and fragmented ceils (lightening arrow), and interlobular hemorrhage (thin arrow). (H&E, x 500).

contains the renal corpuscles which appear as large spherical structure and renal tubules (proximal and distal convoluted tubules). Each renal corpuscle is surrounded by the Bowman's capsule composed of simple squamous epithelial cells. It encloses the urinary space and the capillary tuft of the glumerulus which consists of blood capillaries and masengial cells. The proximal convoluted tubules have narrow lumen with brush borders; they are lined with few cuboidal cells having large spherical nuclei. The distal tubules have wide lumen and large number of cuboidal cells lacking brush borders (Figure 7). The medulla of the normal kidney consists of descending and ascending limbs of Henels loop and collecting tubules. The wall of descending ones is lined with simple squamous epithelium and that of ascending ones is lined with low cuboidal cells (Figure 12).

B- Control groups:

The histological changes of the kidney sections of control groups showed no remarkable changes when compared with normal group.

b- Treated groups:

The effects of IC insecticide were obvious in kidney glomeruli rather than the renal tubules, where kidney cortex sections of animals treated with 1/100 LD_{50} of IC insecticide for 4 weeks showed clear ploriferation, interstitial haemorrhage, and pyknotic nuclei in glomerular tissue. Furthermore, marked dilatations in renal tubules and urinary spaces were observed (Figure 8). In addition the medullary tubules of the kidney showed heavily interlobular hemorrhage (Figure 13). Renal tubules with homogenous cytoplasm, highly vacuolation and limited haemorrhage in kidney cortex were observed in the rats injected with TQ post-IC insecticide for 4 weeks (Figure 9). Moreover, the medullary tubules of the kidney showed sharply shrunken cells with small, flattened and ovoid dark stained nuclei. Interlobular hemorrhage was obviously prominent (Figure 14). Signs of histological improvement could be observed in rats treated with IC post-TQ injection for 4 weeks, as good architecture of renal tubules and glomeruli but the latter restricted into one side, in addition to limitation of hemorrhage (Figure 10). However the medulla showed dilatation in the lumen of renal tubules, pyknotic cells, and interlobular hemorrhage (Figure 15). Kidney cortex sections of animals treated with TQ and IC together for 4 weeks showed renal tubules with homogenous cytoplasm, regular glomeruli, and limited hemorrhage with few pyknotic cells (Figure 11).

DISCUSSION

The liver is the major site of metabolism including detoxification as well as activation of many compounds (Guyton, 1995). Many changes wree observed in the liver after 4 weeks of IC insecticide administration such as congestion in blood sinusoids, vacuolation in the hepatocytes, with degenerated nuclei, congestion and dilation of the central veins as well as aggregations of lymphocytes. These changes were attributed to the fact that hepatocytes play a key role in body metabolism and are greatly affected by the local concentration of any toxic substance (El-Khatib *et al*., 2003). Moreover, the haemorrhage of the liver may be attributed to damage of endothelial lining of blood vessels by IC (Eweis, 1994). Also, inflammatory cells were aggregated and found as differential foci in the liver parenchyma. The inflammatory cells act as a defence mechanism due to irritation of toxic material and necrosed tissue, which may activate the Kupffer cells (Abd-Allah, 1987). The swelling and vacuolation of the cells are most probably due to the retention of fluid inside the hepatocytes resulting in what is known as cloudly swelling or hydropic degeneration which may be due to reduction of the energy necessary for regulation of ion concentration of the cells (Elwi, 1967), or may be related to a mild or short term anoxia (Hruban *et al*., 1972) and may be related to metabolic stress (De Duve & Wattiaux, 1996). Concerning the efficiency of TQ in the reduction of IC toxicity, the study revealed that *Nigella sativa* in the form of TQ provided protection and modulatory effects against IC toxicity as indicated by an improvement of the histological changes which may be related to antioxidant effect of TQ (El-Gendy *et al*., 2007).

The histopathological effects of IC insecticide on rat kidneys included proliferation and damage of the glomerular tufts associated with hemorrhage and vacuolation as well as dilatation of the urinary spaces. The renal tubules showed a wide lumen lined with shrunken cells with flattened or elongated nuclei; the glumerular tubules of the kidney were vacuolated due to oedema. With excessive toxicity concentration, destruction of the glomerular tubules occurred which may be due to degenerative changes resulting from collection of the albuminous material lining during the excretion of the high concentration of the toxin in the urine (Nebbia and Fogliato, 1987). TQ supplementation provided protective and modulatory effect against IC toxicity as indicated by improvement of the histological changes in the cortex and the medulla of the kidney by reducing the oxidative stress which induced inflammation, or by preserving the activity of the anti-oxidant enzymes, as well as its ability to prevent the energy decline in kidney tissues (Mohamed and Nagi, 2007).

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