

Immunohistochemical Detection of Bcl-2 Protein in Normal and Abnormal Rat Liver

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Abstract: Animals treated with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 2, 4 and 6 weeks in the present study revealed many histopathological and immunohistochemical observations in the liver. Examination of liver sections after experimental periods of treatment manifested loss of normal hepatic structure; inflammatory infiltration, marked enlarged vacuolated cytoplasm in cells, congestion of blood vessels, as well as, some of the degenerated cells showed karyorhexis, pyknosis and area of necrosis. the present study revealed negative expression of Bcl-2 proteins in all hepatic cells of control rats and positive expression of Bcl-2 proteins in hepatic cells of treated rats with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper.

Key words: inflammatory infiltration, Bcl-2 proteins and immunohistochemical.

1. INTRODUCTION

The liver is a vital organ, essential for life. Its functions center mostly around taking up molecules and macromolecules from the blood, enzymatically modifying them, and eventually returning them to the bloodstream in different forms for distribution to the body's cells and tissues (1).

Talman (2) reported that hypothyroidism induced by pyrazole (120 mg/100 g body weight for 6 days) significantly depressed the rate of regeneration. Pyrazole caused a specific lesion in the thyroid gland, was used to render rats hypothyroid. The histological appearance and significant increase in mass of the thyroid of the pyrazole-treated rats confirm that the chemical had a deleterious effect on the gland.

The administration of antilipolytic drug (3,5-dimethylpyrazole at dose 12 mg/kg body weight) revealed as early as 30 min many vacuolated lysosomes at the electron microscopic level and autophagic vacuoles are observed in the liver cells after 1 hour (3). Antilipolytic drugs induced both autophagic proteolysis and higher expression of an autophagy related gene. The effect of antilipolytic drug on autophagy gene expression might not be secondary to the stimulation of autophagic proteolysis (4).

2. MATERIAL AND METHODS

2.1 Animals

Healthy adult male albino rats (*Rattus norvegicus*), approximately three months old and weight (120 ± 5) g were used in the present study. The animals were kept under constant condition of temperature for at least two weeks before the experimental period. Animals were maintained on a standard diet, manufactured especially for laboratory purposes, obtained from Atimida Company for national development. Water was available *ad libitum*. Animals were kept under constant temperature (30±2C°) and the humidity was 45±5% with 12:12 light-dark cycle.

2.2 Chemical Used

The ligand 1,5-bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane was prepared following a previously described procedure (5)

1,5-bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane (C₁₈H₂₈N₄O₅-Cu) was prepared by adding a solution of 1,5-bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane (0.262 g, 1 mmol) in 2 ml of acetone to a suspension of Cu(CH₃COO)₂ H₂O (0.199 g, 1 mmol) in 2 ml of the same solvent and stirring

the mixture for 24 hours at room temperature. Green crystals formed were filtered and dried in vacuo.

1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatecopper (C₁₈H₂₈N₄O₅-Cu) was dissolved in 0.9% mammalian saline (9 gm sodium chloride dissolved in 1000 ml distilled water) and injected intraperitoneally (ip) at dose 12 mg/kg body weight /day (6) and (4).

2.3 Experimental Design

The animals were divided into 2 groups.

1- Control group:

Animals of this group (25 rats) were maintained on normal diet throughout the whole experimental period. They were sacrificed after different times parallel to that of treated groups.

2- Group 2:

Animals of this group (24 rats) were injected daily for 6 weeks intraperitoneally (ip) by 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatecopper freshly dissolved in saline (12 mg/kg bw/ day). Animals were then sacrificed 2, 4 and 6 weeks after beginning of the treatment, 8 animals in each period were sacrificed 2 hr after injection.

A- Histological Preparation:

For light microscopic studies, immediately after sacrifice, liver was removed carefully and quickly fixed in 10% neutral formalin for 24 hr, washed in running tap water for 24hr, fixed and stored in 70% ethyl alcohol. Tissue pieces were dehydrated in ascending series of ethyl alcohol (70%, 80%, 90% and two changes 100%), cleared in two changes of xylene and embedded in molten paraplast paraffin (mp. 50-58 oC). Sections of 5 microns thickness were cut using rotary microtome (Leica, Model Rm 2125, Germany), and mounted on clean slides without using any adhesive medium. For histological examination, sections were stained with Ehrlich's haematoxylin and counterstained with eosin (7).

B- Immunohistochemical Detection of Bcl-2 Protein:

The Bcl-2 protein was detected by the immunoenzymatic alkaline phosphatase anti-alkaline phosphatase method, using an anti-human Bcl-2 monoclonal antibody (DAKO A/S, Glostrup, Denmark) (8).

3. RESULTS

A- Histological Observations:

Liver sections of control rats revealed that the hepatocytes arranged in strands around the central vein with one or two spherical nuclei and eosinophilic cytoplasm. Blood sinusoids are occupied by phagocytic Kupffer cells (Figs. 1&2).

Animals treated with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatecopper intraperitoneally at dose 12 mg/kg showed many histological changes throughout the whole experimental periods. After 2 weeks of treatment liver sections exhibited abnormal arrangement of hepatic strands, inflammatory infiltration around widened blood vessel, few condensed cells and degenerated liver cells were observed with enlarged congested blood vessel (Fig. 3).

Examination of liver sections after 4 weeks of treatment showed loss of normal hepatic structure. The bile ductule was surrounded by inflammatory cells and fibrosis with collagen deposition. Few Kupffer cells and widened enlarged sinusoids with condensed cells were seen. Large spaces were detected in some areas due to degeneration of hepatocytes (Fig. 4).

Examination liver sections after 6 weeks of treatment showed severe changes including disarrangement of hepatic strands and loss of normal hepatic structure. Dense lymphocytic infiltration around the central vein and dark stained hepatocytic nuclei indicating cell pyknosis.

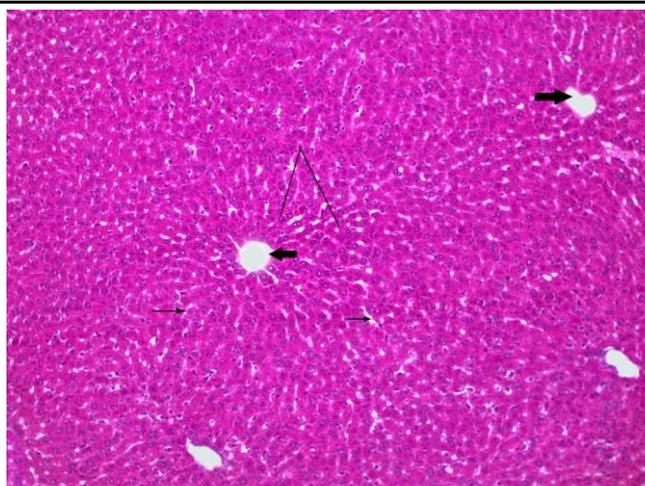


Fig. 1: Sections in the liver of control rats showing the normal hepatic structure; where the hepatocytes (double rod) arranged in strands around the central vein (thick arrow). Blood sinusoids (thin arrow) were also evident (H&E stain, X100).

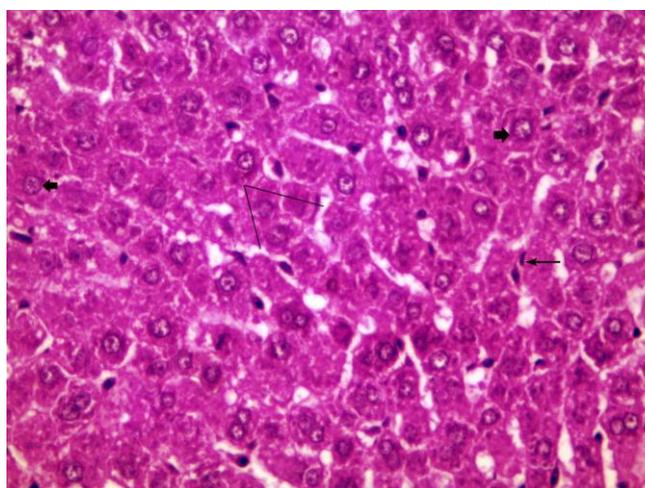


Fig. 2: sections of liver sections of control rats showing; the hepatocytes with eosinophilic cytoplasm and centrally located nuclei (thick arrow). Between the cords of hepatocytes often seen blood sinusoids (double rod) with phagocytic Kupffer cells (thin arrow) (H&E stain, X400).

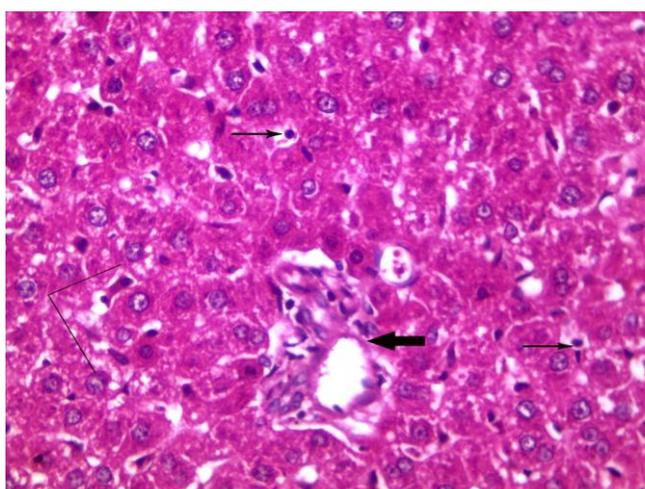


Fig.3: Sections in the liver of rats treated with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 2 weeks showing inflammatory infiltration around widened blood vessel (thick arrow), few condensed cells (thin arrows) and degenerated cells (double rod) (H&E stain, X400).

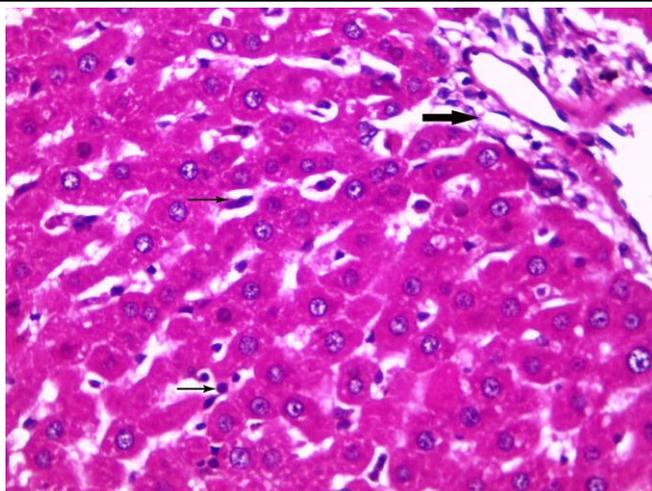
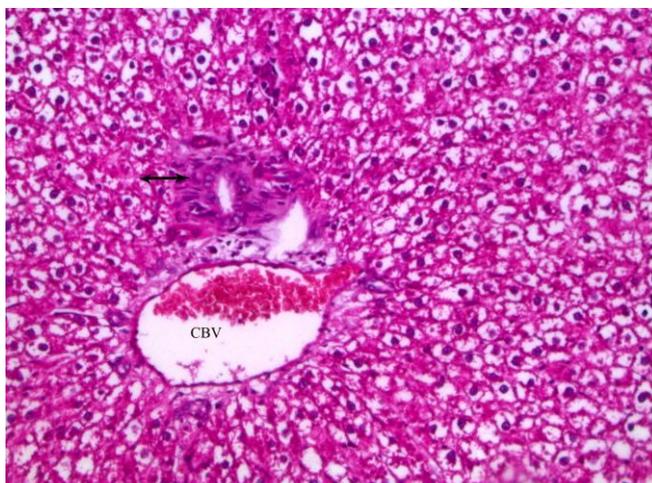
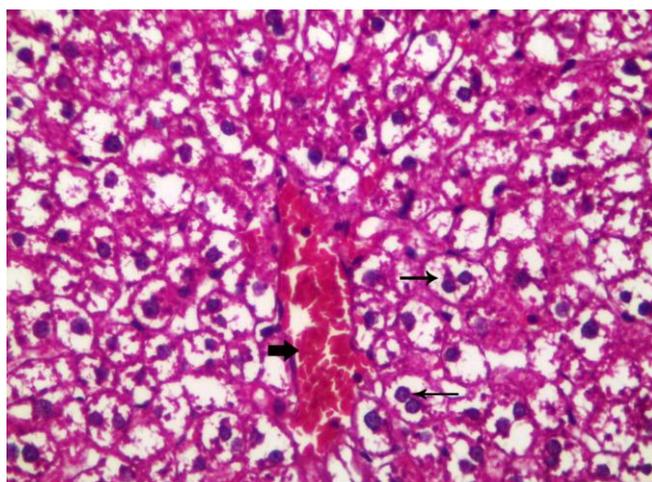


Fig.4: Sections in the liver of rats treated with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 4 weeks showing inflammatory infiltration around widened portal vein and bile ductule (thick arrows), widened enlarged sinusoids with condensed cells (thin arrow) (H&E stain, X400)



(a)



(b)

Fig. 5: Sections in the liver of rats treated with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 6 weeks showing loss of normal hepatic structure. Most of cells had condensed nuclei (pyknotic cells) with marked cytoplasmic vacuolation. Congested blood vessel with hemorrhage (CBV) (thick arrow), inflammatory infiltration around widened bile ductule (double head arrow) and binucleated cells (thin arrows) (H&E stain, (a) X200 & (b) X400).

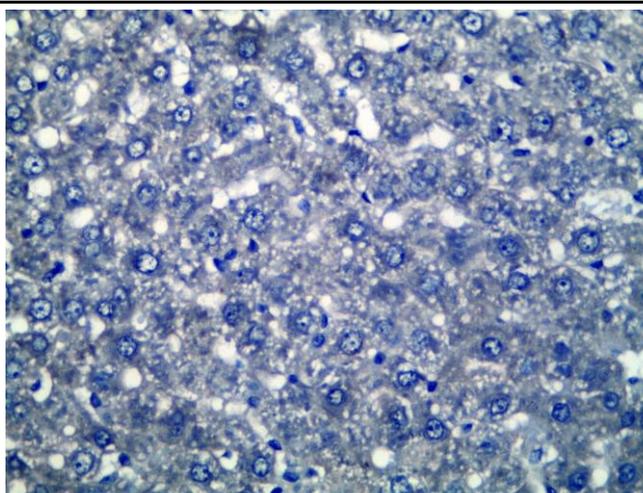


Fig. 6: Sections in the liver of control rats showing normal expression of Bcl-2 proteins, negative expression in all hepatic cells (alkaline phosphatase anti-alkaline phosphatase method, X400).

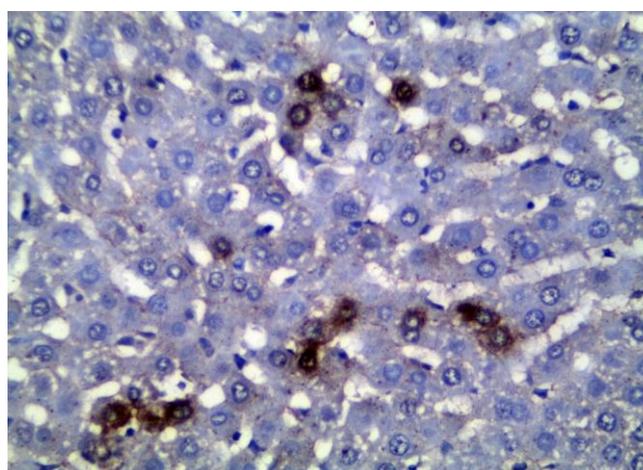


Fig. 7: Sections in the liver rats treated with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 2 weeks showing positive expression of Bcl-2 proteins in some hepatic cells (alkaline phosphatase anti-alkaline phosphatase method, X400).

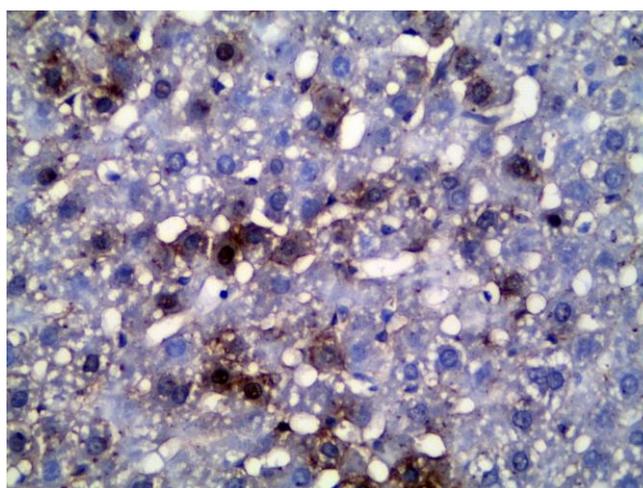


Fig. 8: Sections in the liver rats treated with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 4 weeks showing positive expression of Bcl-2 proteins in some hepatic cells (alkaline phosphatase anti-alkaline phosphatase method, X400).

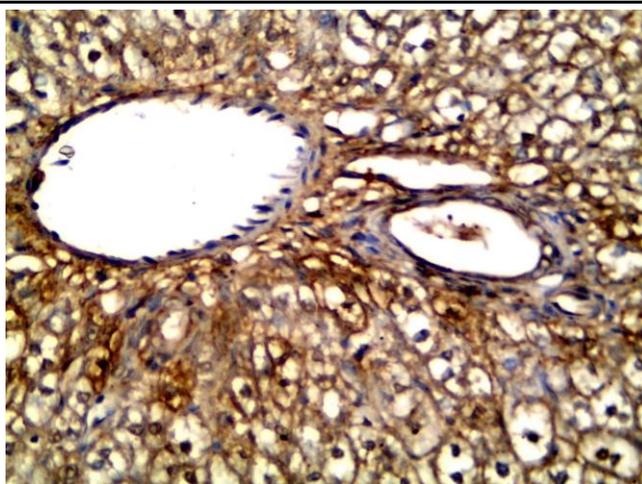


Fig. 9: Sections in the liver rats treated with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 6 weeks showing positive expression of Bcl-2 proteins in all hepatic cells (alkaline phosphatase anti-alkaline phosphatase method, X400).

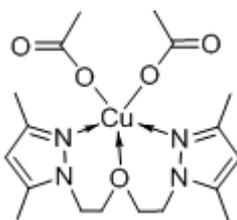
B- Immunohistochemical Observations:

Liver sections of control rats revealed that the Bcl-2 protein was expressed by lymphocytes, epithelial cells lining bile ductules and small interlobular bile ducts, but not by cells lining larger bile ducts. Bcl-2 staining was expressed as small brown-yellow staining in cytoplasm (fig. 6).

Liver sections obtained from rats treated with 1, 5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for different treatments showed that Bcl-2 was expressed by hepatic cells (Figs. 7-9).

4. DISCUSSION

Complex 1,5-bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper was prepared by the reaction of ligand and copper (II) acetate monohydrate in acetone solution similarly to previously reported procedure for copper (II) nitrate complexes (9). The proposed structure for this compound was confirmed by UV-Vis and IR spectroscopy, molar conductivity measurements and elemental analysis data.



1,5-bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper

Our results indicated that treating rats with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper caused many histopathological alterations in the liver structures, inflammatory infiltration, swelling and marked enlarged vacuolated cytoplasm in cells, congestion of blood vessels, hemorrhage, pyknotic cells and binucleated cells, as well as, some of the degenerated cells showed karyorrhexis, pyknosis and area of necrosis. Large spaces were detected in some areas due to degeneration of cells. Wide spread area of necrosis and hyperplasia. Similarly, In human beings primary toxicity of pyrazole was found in the liver, kidney and bone marrow (10). Livers of rats dying 6 to 11 days after the administration of pyrazole showed extensive centrilobular necrosis with inflammatory reactions in the parenchyma as well as fatty infiltration of the surviving cells (11).

Ritva (12), reported that centrilobular necrosis was observed among rats treated with pyrazole at dose 200 mg/kg. The microsomes from the pyrazole-treated animals, the enzyme activity of liver was about 3 times higher than the values obtained for the control microsomes. The microsomes from the pyrazole-treated animals gave a prominent increase in the number of mutations. Rats

injected intraperitoneally with pyrazole at dose 200 mg/kg body wt, once per day for 2 days, pyrazole produced swelling of mitochondria and induced liver histopathology & liver injury (13).

Known effects of antilipolytic agents (3,5-dimethylpyrazole at dose 12 mg/kg body weight) may be related to features of rat liver autophagy (14). The administration of antilipolytic drug (3,5-dimethylpyrazole at dose 12 mg/kg body weight) revealed as early as 30 min many vacuolated lysosomes at the electron microscopic level and autophagic vacuoles are observed in the liver cells after 1 hour. After 1 hr and 45 min, vacuoles often contain recognizable peroxisome (3).

Autophagy is a process involved in cell maintenance could be induced by antilipolytic drugs (eg., 3,5-dimethylpyrazole at dose 12 mg/kg body weight) (15).

Mice injected with pyrazole (150 mg/kg, ip) daily for 2 days, followed by Either (LPS) injection (4 mg/kg, ip). pyrazole enhanced liver injury, oxidative stress, indication of pathological changes, apoptosis, necrosis, positive staining with apoptotic morphology casually appeared at 3 hours, increased at 8 hours that induced by LPS treatment. Single necrotic cells were casually observed at 3 hours, some small necrotic foci were seen at 8 hours, but widespread small necrotic foci and large necrotic areas were seen at 24 hours (16).

In the present work, control liver section showed that, the Bcl-2 protein was expressed by epithelial cells lining bile ductules and small interlobular bile ducts, but not by cells lining larger bile ducts. There was no evidence of Bcl-2 protein expression by hepatocytes. These results are in agreement with that obtained by some investigators. Normal liver sections showed that, Bcl-2 was expressed by bile ductules, but not by hepatocytes or large bile duct epithelium, similar to that recently mentioned in normal adult liver (17) and in fetal liver (18).

In the present work, treating rats with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatecopper caused marked Bcl-2 staining of all liver cells. Similarly, during chemically induced hepatocarcinogenesis in rat, stem cells may be stimulated and give rise to oval cell proliferation in periportal areas (19). Lindros (20) reported that chronic treatment rats with the higher dose of 4-methylpyrazole seemed to cause a relative decrease in the liver protein content.

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