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HISTOCHEMICAL AND HISTOPATHOLOGICAL OBSERVATIONS ON LIVER RAT TREATED WITH 1,5-*BIS*(3,5-DIMETHYLPYRAZOL-1-YL)-3-OXAPENTANE-DIACETATOCOPPER

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The present study provides evidence that 1,5-bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper has an antidiabetic effect, as hypoglycemic agent and as antilipolytic agent, but with many abnormalities. Rats 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 alterations in the liver; inflammatory infiltration, 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 karyorhexis, pyknosis and area of necrosis. The present study revealed significant decrease in proteinic contents in the liver.

Ключевые слова: proteinic contents, pyknosis and necrosis

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].

3,5-Dimethylpyrazole (DMP) as a hypoglycemic agent (one of various agents that decrease the level of glucose in the blood and are used in the treatment of diabetes mellitus) and as antilipolytic agent (one of agents that inhibits lipolysis). 3,5-dimethylpyrazole markedly depressed plasma fatty acid (FFA) and blood sugar after 15 minutes to 3 hours of its administration [2].

Pyrazole administration increased the toxicity of dimethylnitrosamine when measured as a 50% lethal dose or as a histopathological effect on the liver [3].

MATERIAL AND METHODS

Animals

Healthy adult male albino rats (*Rattus norvegius*), 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.

Chemical used

The ligand 1,5-*bis*(3,5-dimethylpyrazol-1-yl)-3-oxapentane was prepared following a previously described procedure [4]

1,5-*bis*(3,5-dimethylpyrazol-1-yl)-3-oxapentane ($C_{18}H_{28}N_4O_5$-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 $Cu(CH_3Coo)_2$ H_2O (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-diacetatocopper ($C_{18}H_{28}N_4O_5$-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 [5].

EXPERIMENTAL DESIGN

The animals were divided into 2 groups.

1- *Control group:*

Animals of this group (24 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-diacetatocopper 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 sacrification, 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 °C). 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 [6].

B. Histochemical procedures:

For histochemical purposes, small pieces of liver were fixed in 10% neutral formalin for demonstration of total protein. Sections of 5 microns thickness were cut. Total proteins demonstrated by mercury bromophenol blue method [7].

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.

Animals treated with 1,5-*bis*(3,5-dimethylpyrazol-1-yl)-3-oxapentane-diacetato-copper intraperitoneally at dose 12 mg/kg showed many histological abnormalities 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, binucleated cells and degenerated liver cells were observed with enlarged congested blood vessel. Moreover, slight widened blood sinusoids, slight cytoplasmic vacuolation, activated enlarged Kupffer cells and fatty degeneration indicated by large number of fatty droplets with different size was observed.

Examination of liver sections after 4 weeks of treatment manifested loss of normal hepatic architecture. Infiltration of inflammatory cells with abnormal mitosis in inflammatory area was observed. The bile ductule was surrounded by inflammatory cells and fibrosis with collagen deposition. Few Kuppfer cells and widened enlarged sinusoids were seen. Congested blood vessel and increase in central vein size with hemorrhage were observed. Steatosis, condensed cells and binucleated cells were seen. Large spaces were detected in some areas due to degeneration of hepatocytes.

After 6 weeks following the same previous treatment, liver sections examination 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. Swelling and marked enlarged vacuolated cytoplasm in parenchymal cells with condensed nuclei were noticed. Congested blood vessel with hemorrhage, pyknotic cells and binucleated cells. Some of the degenerated cells showed karyorhexis and other degenerated cells lost their boundaries. Wide spread area of necrosis and hyperplasia within the liver lobules and around central veins and the portal tracts. Most cells showed nuclei with signs of karyolysis, pyknosis and area of necrosis.

B- Histochemical observations (Total proteins):

Hepatocytes of control rats contains excessive amount of total proteins in the form of fine blue granules of various sizes randomly distributed in cytoplasm. The plasma membranes, the nuclear envelopes, walls of blood vessels, as well as both chromatin bodies and nucleoli exhibiting deep colouration, while Kupffer cells and endothelial lining cells of sinusoids give moderate reactivity with bromophenol blue.

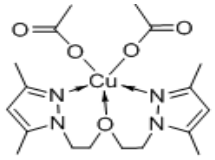
Gradual reduction of proteinic contents in liver following treatment with 1,5-*bis*(3,5-dimethylpyrazol-1-yl)-3-oxapentane-diacetato-copper throughout different experimental periods. In most hepatocytes, the positively stained protein granules were observed perinuclear or peripheral in position and were exhibiting a blue coloration, while the rest of the cytoplasm appeared vacuolated, the nuclei and plasma membrane were intensely stained.

DISCUSSION

Complex 1,5-*bis*(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper was prepared by the reaction of ligand and copper [11] acetate monohydrate in acetone solution similary to previously reported procedure for copper [11] nitrate complexes [8]. The proposed structure for this compound was confirmed by UV-Vis and IR spectroscopy, molar conductivity measurements and elemental analysis data.

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 karyorhexis, 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 [9]. Livers of rats dying 6 to 11 days after the administration of pyrazole showed extensive centrolobular necrosis with inflammatory reactions in the parenchyma as well as fatty infiltration of the surviving cells [10].



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

Ritva [3], 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 [11].

Known effects of antilipolytic agents (3,5-dimethylpyrazole at dose 12 mg/kg body weight) may be related to features of rat liver autophagy [12, 13]. 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 [14].

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, indicatation 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].

Results obtained in the present work revealed that treating rat with 1,5-*bis*(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper caused reduction in total proteins contents in tissue of liver. These results are in agreement with that obtained by some investigators. Similarly, the administration of antilipolytic agents (eg., 3,5-dimethylpyrazole at dose 12 mg/kg body weight) can trigger autophagy and intracellular degradation of proteins in rat liver cells. Autophagic vacuoles are formed in a few minutes and their number increases thereafter to reach the highest values in a few hours [14].

The administration of rat by antilipolytic drugs (3,5-dimethylpyrazole at dose 12mg/Kg body weight) revealed that the peroxisomal enzyme activities decreased significantly in 2-3 hours which play an important role in the control of lipid metabolism and also increased degradation of liver proteins [17].

Mice injected with pyrazole (150 mg/kg, ip) daily for 2 days, followed by Either (LPS) injection (4 mg/kg, ip). pyrazole enhanced positive TUNEL staining that induced by LPS treatment [16].

The antilipolytic agent 3,5-dimethylpyrazole at dose 12 mg/kg body weight can increase rat liver protein degradation. DMP treatment caused an increase in the expression of the LC3 gene (whose product is one of the best known markers of autophagy) [5].

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TOXICOLOGICAL ASSESSMENT OF EFFECT OF 1,5-BIS(3,5-DIMETHYLPYRAZOL-1-YL)-3-OXAPENTANE-DIACETATOCOPPER ON ALBINO RAT LIVER

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The present study provides evidence that 1,5-Bis (3,5-dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper has a toxicological and histopathological effect on the liver. The present study revealed many histopathological alterations on the liver; inflammatory infiltration, marked vacuolated cytoplasm, congested blood vessels, hemorrhage, pyknotic and binucleated cells, as well as, some of the degenerated cells showed karyorhexis, pyknosis and necrosis. Sera of animals treated with 1,5-Bis (3,5-dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper revealed a significant decrease in glucose and albumin.

Ключевые слова: histopathological alterations, glucose and albumin.

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].

3,5-Dimethylpyrazole as a hypoglycemic agent (one of various agents that decrease the level of glucose in the blood and are used in the treatment of diabetes mellitus) and as antilipolytic agent (one of agents that inhibits lipolysis). 3,5-Dimethylpyrazole markedly depressed plasma fatty acid and blood sugar after 15 minutes to 3 hours of its administration [2]. The administration of antilipolytic drugs 3,5-dimethylpyrazole to rats induced significant decrease in plasma fatty acid in 15 min, glucose and insulin [3].

The administration of rat by antilipolytic drugs (3,5 dimethylpyrazole at dose 12mg/Kg body weight) revealed that the peroxisomal enzyme activities decreased significantly in 2-3