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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

hours which increased degradation of liver proteins [4].

Bergamini *et al.* [5], showed similar results of elevated activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) when rats administered with 3,5-dimethylpyrazole at dose 12 mg/kg body weight. Either LPS-induced liver injury in mice was enhanced by pyrazole, as indicated by pathological changes and increases in ALT and AST. LPS-induced oxidative stress was also enhanced by pyrazole [6].

Treatment of rats with pyrazole elevated the hepatic microsomal dimethylnitrosamine demethylase activity (DMNd) by several fold [7]. Pyrazole administration increased the toxicity of dimethylnitrosamine when measured as a 50% lethal dose or as a histopathological effect on the liver [8].

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 [4]. Antilipolytic drugs induced both autophagic proteolysis and higher expression of an autopagy related gene. The effect of antilipolytic drug on autophagy gene expression might not be secondary to the stimulation of autophagic proteolysis [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±2Cº) 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 [9].

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-oxapentanediacetatocopper ($C_{18}H_{28}N_4O_5$-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 [3].

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-oxapentanediacetatocopper 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.

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, 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 [10].

Biochemical analysis

Blood samples were taken from portal vein and left to coagulate at 37ºC in incubator, centrifugated at 174 g/min for 15 min (1550 r.p.m) with centrifuge (Shanghai Surgical Instruments Factory, Model 9-1). Sera were stored at -20Cº until further analysis. Specimens from control and all treated groups were obtained for examination after 2, 4 and 6 weeks from starting the experiment.

Serum albumin

Serum albumin was determined according the method of Doumas *et al.* [11].

Serum glucose

Serum glucose was determined according the method of Bergmeyer *et al.* [12].

Statistical analysis

All biochemical results were expressed as mean ± standard error. The results were analyzed statistically utilizing computer program (Excel) with two levels of significance at P≤0.05 & P≤0.01 denote low and high significant changes from control, respectively.

RESULTS

Histological observations

The hepatocytes of control rats 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. Liver sections after 2 weeks of treatment, 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 were.

Liver sections after 4 weeks of treatment manifested loss of normal hepatic architecture. Inflammatory infiltration was observed, the bile ductule was surrounded by inflammatory cells and fibrosis with collagen deposition. Few Kupffer 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 also were seen. Large spaces were detected in some areas due to degeneration of hepatocytes.

Liver sections after 6 weeks of the same previous 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. 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 necrosis.

Biochemical results

Serum Albumin (g/dL).

Intraperitoneal administration of 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper to rats revealed that biochemical determination of albumin after 2, 4 and 6 weeks of injection clearly exhibited significant decrease compared with control group.

Serum glucose (mg/dl).

Intraperitoneal administration of 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentanedi-acetatocopper to rats revealed that biochemical determination of serum glucose after 2, 4 and 6 weeks of injection clearly exhibited significant decrease compared with control group.

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 similary to previously reported procedure for copper nitrate complexes [13]. 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, 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 [14].

In human beings primary toxicity of pyrazole was found in the liver, kidney and bone marrow [15]. 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 [16].

Known effects of antilipolytic agents (3,5-dimethylpyrazole at dose 12 mg/kg body weight) may be related to features of rat liver autophagy [17]. 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].

Examination of sera of animals treated with 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper in the present study revealed a significant decrease in serum glucose and albumin. Several investigators also obtained similar results. In this concern, treatment of fasting rats with antilipolytic drugs (either 3,5-dimethylpyrazole at dose 12 mg/kg body weight) resulted in a decrease in free fatty acid and glucose plasma levels within 5-10 min and in a significant increase in the plasma glucagon to insulin ratio within 15 min. [18]. The administration of antilipolytic drugs (3,5-dimethylpyrazole at dose 12 mg/Kg body weight) decreased the concentration of free fatty acids, glucose and insulin in rat blood plasma (by 10-15 min), and peroxisomal enzyme activities decrease significantly in 2-3 hours which play an important role in the control of lipid metabolism [2].

Stem cells may be stimulated and give rise to oval cell proliferation in periportal areas, during chemically induced hepatocarcinogenesis in rat [19]. Lindros *et al.* [20] reported that chronic treatment rats with the higher dose of 4-methylpyrazole seemed to cause a relative decrease in the liver protein content.

The administration of antilipolytic agents (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 [3].

In conclusion, 1,5-bis (3,5-dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper has a toxicological effect in blood and liver of albino rats.

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