



## **EFFECT OF 1,5-BIS(3,5-DIMETHYLPYRAZOL-1-YL) -3-OXAPENTANE-DIACETATOCOPPER ON PANCREAS OF ALBINO RAT**

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### **ABSTRACT**

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 pancreas; inflammatory infiltration, marked enlarged vacuolated cytoplasm in pancreatic cells, congestion of blood vessels, 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 pancreas.

**Keywords:**proteinic contents, pancreas, pyknosis and necrosis.

## INTRODUCTION

The pancreas is an organ composed of endocrine and exocrine tissue. The endocrine portion is the islets of Langerhans, contains cells that secrete insulin and glucagon hormones. The exocrine pancreas is a complex gland which contains many secretory acini which produce and release digestive enzymes. Insulin is hormone secreted by the islets of Langerhans in the pancreas into the blood when animals eat and the glucose level in the blood increases. Insulin stimulates the uptake, storage and use of glucose by tissues. These activities result in a decrease in the level of blood glucose (Butler, 1995).

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. The mechanism of hypoglycemic activity of 3,5-dimethylpyrazole is not the same as insulin. The action 3,5-dimethylpyrazole is similar to that of insulin in that; It increases glucose oxidation and decreases plasma free fatty acids of intact rats as reported for insulin and decrease blood glucose. 3,5-dimethylpyrazole is unlike insulin in that; It is not effective in lowering blood sugar of eviscerated rats which respond to insulin (George and William, 1965).

Hypoglycaemia and stimulation of the endocrine pancreas of rats after 3,5-dimethylpyrazole administration at dose 12 mg/kg body weight were observed (Locci and Bergamini, 1983).

The antilipolytic agent 3,5-dimethylpyrazole inhibits insulin release in response to both nutrient secretagogues and cyclic adenosine monophosphate (cAMP) agonists in isolated rat islets (Masiello *et al.*, 2002).

## MATERIAL AND METHODS

### 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.

### Chemical used:

The ligand 1,5-bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane was prepared following a previously described procedure (Potapov *et al.*, 2007)

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  $Cu(CH_3COO)_2 \cdot H_2O$  (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 (Donatiaet al., 2008).

## 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-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 sacrifice, pancreas 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 (Lillie and Fulmer, 1976).

#### B- Histochemical procedures:

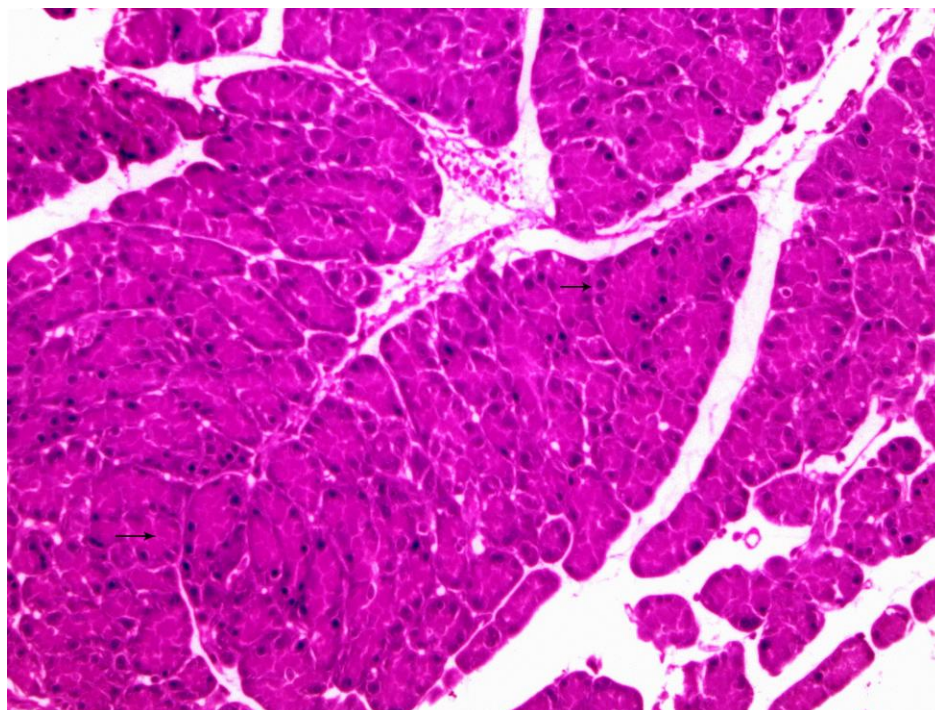
For histochemical purposes, small pieces of pancreas were fixed in 10% neutral formalin for demonstration of total protein. Sections of 5 microns thickness were cut. Total proteins demonstrated by mercury bromophenol blue method (Pearse, 1972).

## RESULTS

### A- Histological observations:

#### 1- Control group:

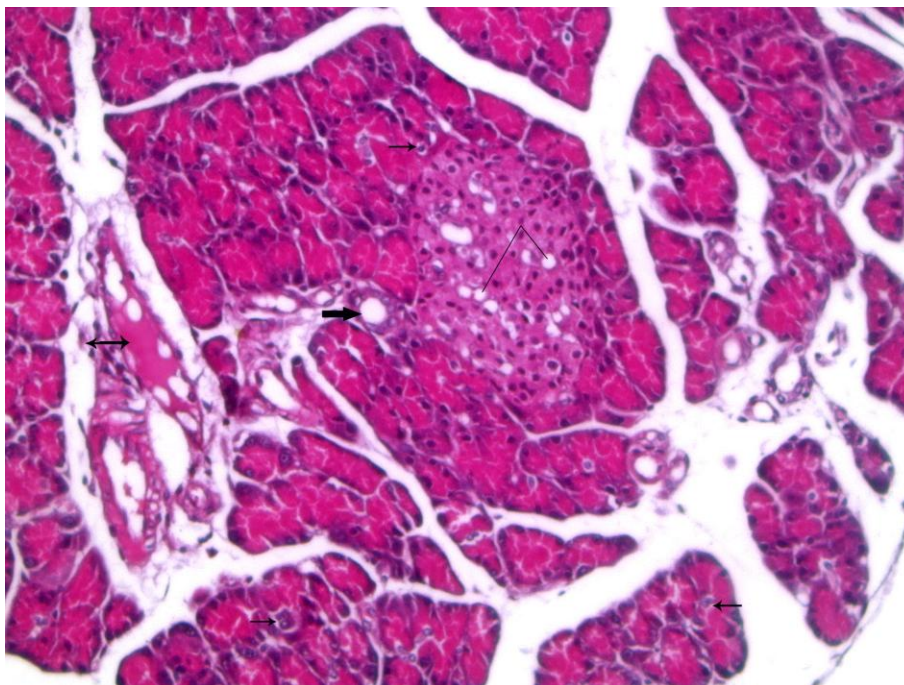
The pancreas is an organ composed of endocrine and exocrine tissue. The endocrine portion is the islets of langerhans, contains cells that secrete insulin and glucagon hormones. The exocrine pancreas is a complex gland which contains many secretory units called acini arranged around a system of ducts. The ducts combine to form the large pancreatic duct. The pancreas contains thousands of acini, each acinus composed of pyramid - shaped secretory cells (acinar cells) which produce and release digestive enzymes. The nucleus of the acinar cell is located at the base of the cell. (fig. 1).



**Figure 1:** Sections in the pancreas of control rats showing the normal pancreatic structure; where the nucleus of the acinar cell is located at the base of the cell (thin arrow). The islets of langerhans (thick arrow) were also evident (H&E stain, X200)

#### 2- Treated groups:

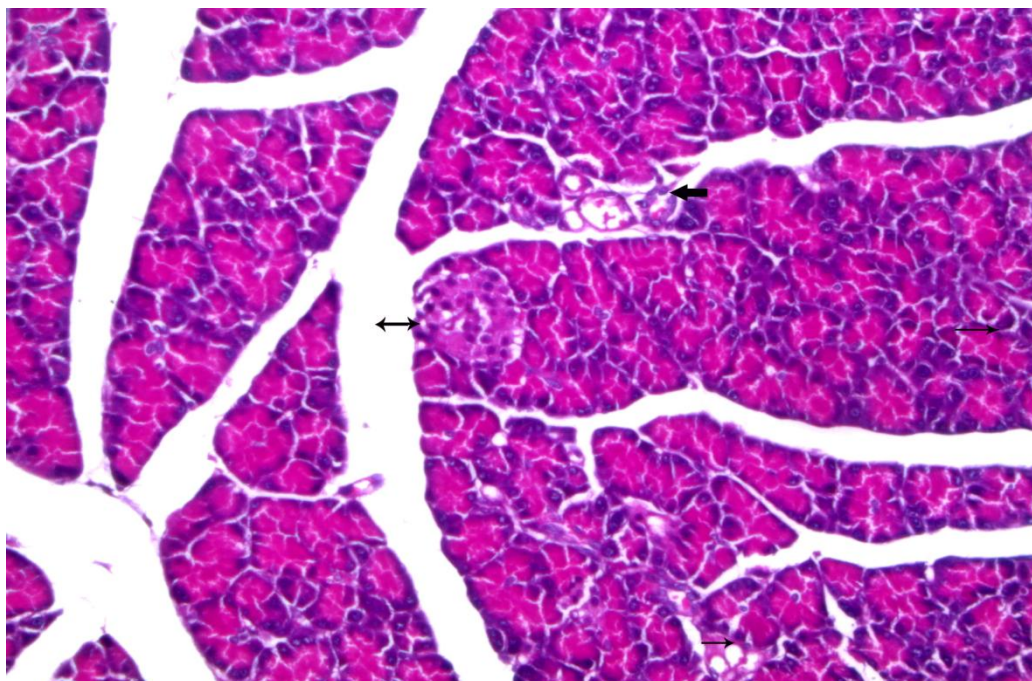
Rats treated with 1,5-Bis (3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper exhibited many histopathological changes in pancreas. After 2 weeks of treatment pancreatic sections showed inflammatory infiltration around widened pancreatic duct. Mild congestion, mild edema and haemorrhage of blood vessels were observed. Degeneration and vaculation of the acinar cells and the islets of langerhans were also evident (fig. 2).



**Figure 2:** Sections in the pancreas of rats treated with 1,5-Bis (3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 2 weeks showing inflammatory infiltration around widened pancreatic duct (thick arrows) and blood vessels with haemorrhage (double head arrow). Degeneration and vacuolation of the acinar cells (thin arrow) and the islets of langerhans (double rod) were also evident (H&E stain, X200)

After 4 weeks of treatment pancreatic sections showed marked inflammatory infiltration around widened pancreatic duct and blood vessels with edema and haemorrhage. Marked degeneration, vacuolation and karyolysis of the acinar cells and the islets of langerhans were also observed (fig.3).

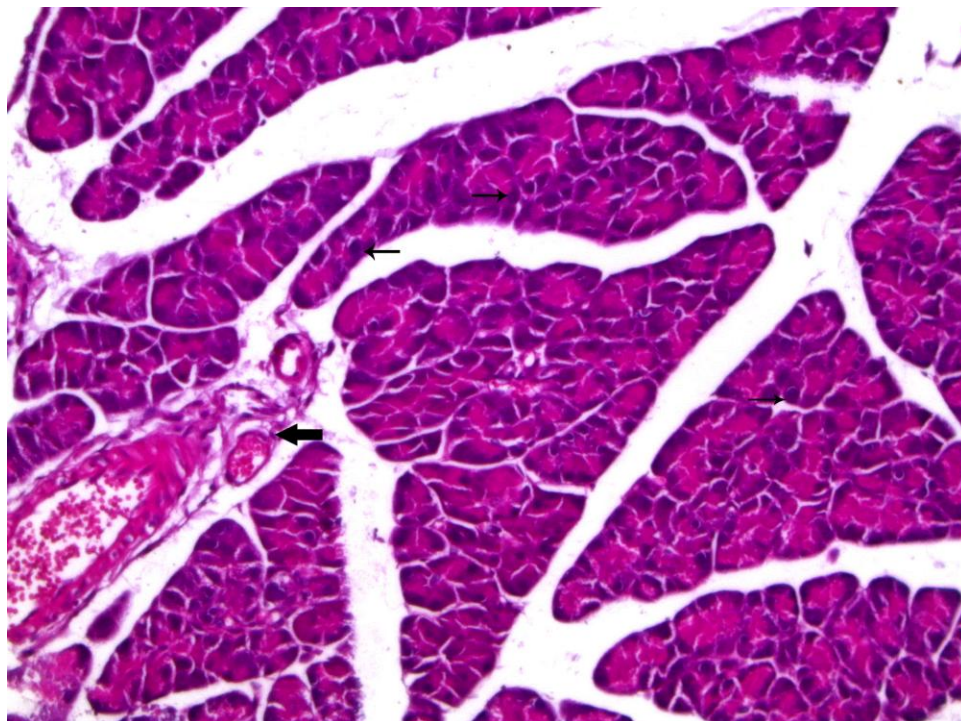




**Figure**

**3:** Sections in the pancreas of rats treated with 1,5-Bis (3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 4 weeks showing inflammatory infiltration around widened pancreatic duct and blood vessels with edema and haemorrhage (thick arrows). Marked degeneration, vaculation and karyolysis of the acinar cells (thin arrow) and the islets of langerhans (double rod & head arrow) were also evident (H&E stain, X200)

Examination of pancreatic sections after 6 weeks of treatment showed fatty degeneration indicated by large number of fatty droplets, increased congestion of blood vessels with haemorrhage. Marked degeneration, vaculation and loss of pancreatic acinar cells, and the spaces between the islets and ducts enlarged and became filled with fibroblast-like cells. Most of cells had condensed nuclei (pyknotic cells) with marked cytoplasmic vaculation and lost normal pancreatic structure (fig.4).



**Figure 4:** Sections in the pancreas of rats treated with 1,5-Bis (3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 6 weeks showing marked inflammatory infiltration around widened pancreatic duct and blood vessels with edema and haemorrhage (thick arrows). Marked degeneration, vacuolation and pyknosis of the acinar cells and lost normal pancreatic structure (thin arrow) were also evident (H&E stain, (a) X200 & (b) X400)

## **B- Histochemical observations (Total proteins):**

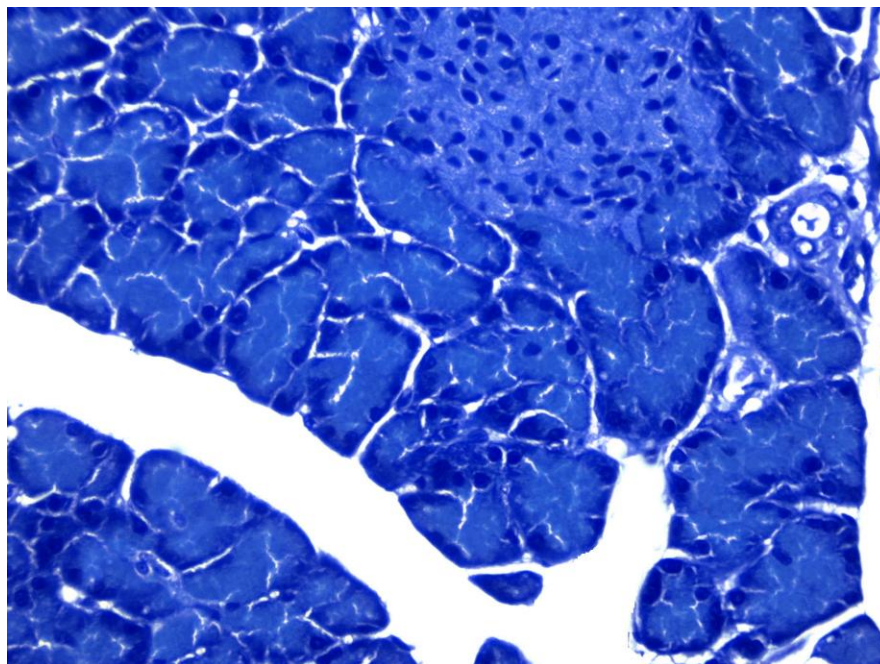
### **1- Control group:**

Examination of pancreas of control rats showed a strong positive reaction of the cytoplasm and nucleus of pancreatic cells, pancreatic ducts and the islets of langerhans stained with mercury bromophenol blue technique (fig.5).

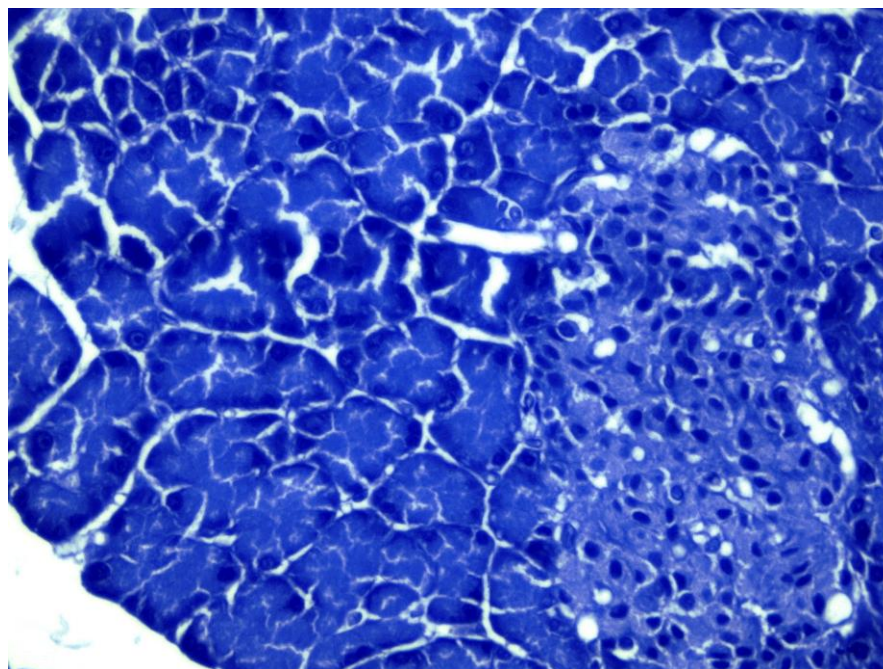
### **2- Treated groups:**

Gradual reduction of proteinic contents in the pancreas following treatment with 1,5-Bis (3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper throughout different experimental periods (figs.6-8).



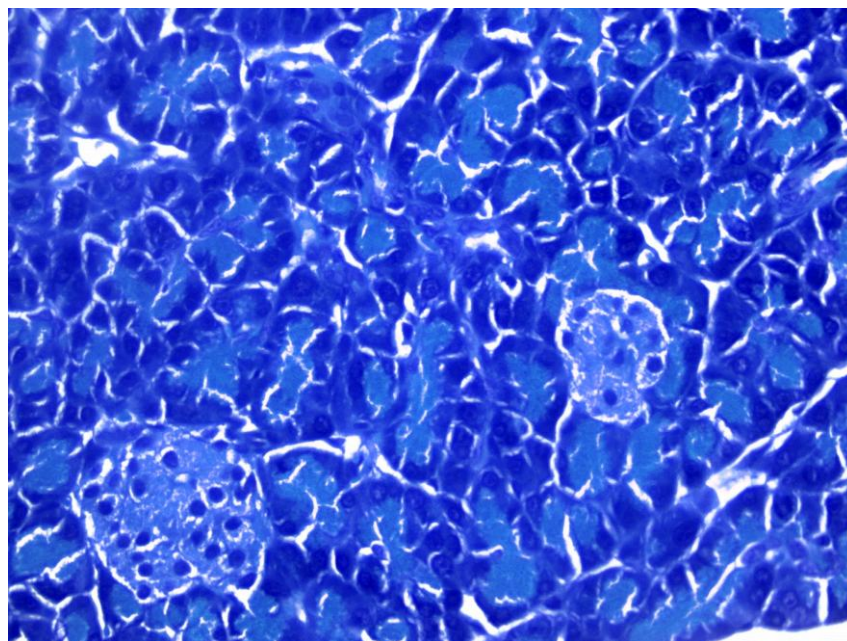


**Figure 5:** Sections in the pancreas of control rats showing normal distribution of total proteins (Bromophenol blue stain, X400)

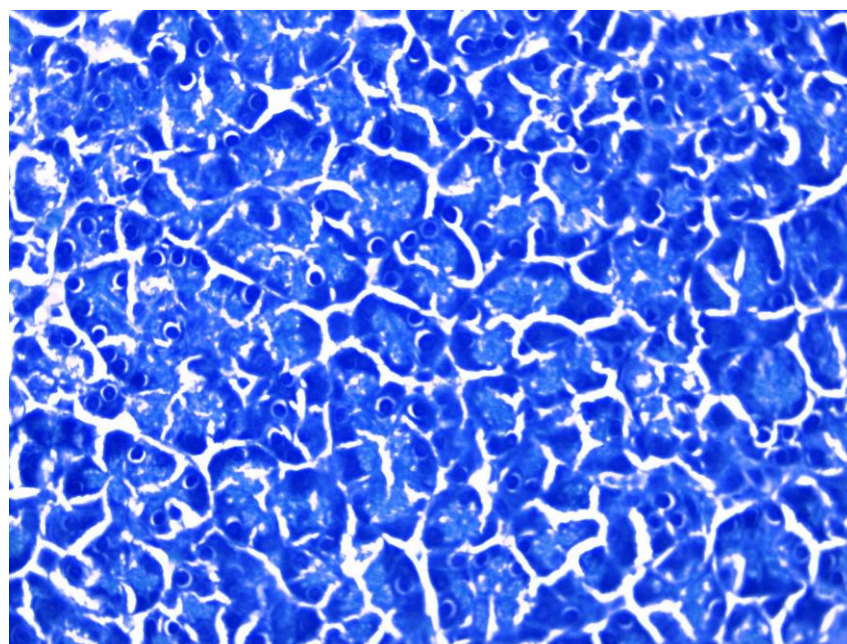


**Figure 6:** Sections in the pancreas of rats treated with 1,5-Bis (3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 2 weeks showing decrease in the amount of protein content (Bromophenol blue stain, X400)





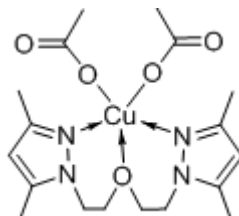
**Figure 7:** Sections in the pancreas of rats treated with 1,5-Bis (3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 4 weeks showing decrease in the amount of protein content (Bromophenol blue stain, X400)



**Figure 8:** Sections in the pancreas of rats treated with 1,5-Bis (3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 6 weeks showing marked decrease in the amount of protein content (Bromophenol blue stain, X400)

## 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 (Potapov *et al.*, 2009). 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 pancreas structures, inflammatory infiltration, swelling and marked enlarged vacuolated cytoplasm in pancreatic cells, congestion of blood vessels with edema and haemorrhage, 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. Similarly, Yamada *et al.* (1987) suggested that the ethanol-induced pancreatic injury in rat, even at an early stage, accelerates the collagen metabolism in the pancreas. These findings were also confirmed in ethanol-pyrazole treated rats.

In human beings primary toxicity of pyrazole was found in the liver, kidney and bone marrow (Wilson and Bottiglieri, 1962). 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 (Lelbach, 1969).

Ritva *et al.* (1983), reported that centrilobular necrosis was observed among rats treated with pyrazole at dose 200 mg/kg. 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 (Yongkeet *et al.*, 2005).

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 (Lu and Cederbaum, 2006).

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 pancreas. These results are in agreement with that obtained by some investigators. Similarly, The antilipolytic agent 3,5-dimethylpyrazole at dose 12 mg/kg body weight can increase rat liver protein degradation (Donatiet *al*, 2008).

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 (Bergaminiet *al*,1987).

The administration of rat by antilipolytic drugs (3,5dimethylpyrazole 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 (Bergamini and Segal, 1987 ).

## REFERENCES

1. Bergamini, E. and Segal, H.L. (1987): Effects of Antilipolytic drugs on hepatic peroxisomes. *Biol. and Med.*, 295-303.
2. Bergamini, E.; Tata, V. D.E.; LoccrCubeddu, T., Masiello, P. and Pollera, M. (1987): Increased degradation in rat liver induced by antilipolyticagents:A model for studying autophagy and protein degradation in Liver. *Exp. and mol. pathol.*, 46: 114- 122.
3. Butler, L.K. (1995): Regulation of blood glucose levels in normal and diabetic rats.Texas-Austin:181-202.
4. Donatia, A.; Ventrutia, A.; Cavallinia,G.; Masinia, M.; Vittorinia, S.; Chantretb, I.; Codognob, P.; and Bergamini, E. (2008): In vivo effect of an antilipolytic drug (3,5-dimethylpyrazole) on autophagic proteolysis and autophagy-related gene expression in rat liver. *Biochem. andBiophys. Res. Commun.*, 366 : 786–792.
5. George, C. G. and William E. D. (1965): Effect of a New Hypoglycemic Agent, 3,5-Dimethylpyrazole, on Carbohydrate and Free Fatty Acid Metabolism. *Diabetes.* (14) 8: 507-515.
6. Lelbach, W.K. (1969): Liver cell necrosis in rats after prolonged ethanol ingestion under the influence of an alcoholdehydrogenase inhibitor. *Experientia*, 25: 816-818.
7. Lillie, R.D. and Fulmer, H.M. (1976): *Histopathological Technique and Practical Histochemistry.* 4<sup>th</sup>edn. New York, McGraw Hill.
8. Locci C. T. and Bergamini, E. (1983): Effects of antilipolytic agents on peroxisomal beta-oxidation of fatty acids in rat liver.*Biochem. Pharmacol.*, (32)11: 1807-1809.



9. Lu, Y. and Cederbaum, A. I. (2006): Enhancement by pyrazole of lipopolysaccharide-induced liver injury in mice: role of cytochrome P450 2E1 and 2A5. *Hepatology*, (44)1:263-274.
10. Masiello, P.; Novelli, M.; Bombara, M.; Fierabracci, V.; Vittorini, S.; Prentki, M. and Bergamini, E. (2002): The antilipolytic agent 3,5-dimethylpyrazole inhibits insulin release in response to both nutrient secretagogues and cyclic adenosine monophosphate agonists in isolated rat islets. *Met.*, (51)1:110-114.
11. Pearse, A. G. E. (1972): *Histochemistry, Theoretical and Applied*, 3<sup>rd</sup>ed., vol. 2. Churchill Livingstone. London.
12. Potapov, A. S.; Domina, G. A.; Domina, G. A.; Khlebnikov, A. I. и Ogorodnikov, V. D. (2007): Facile synthesis of flexible Bis(pyrazol-1-yl) alkane and related ligands in a superbasic medium // *Eur. J. Org. Chem.*; 5112-5116.
13. Potapov, A. S.; Nudnova, E. A.; Domina, G. A.; Kirpotina, L. N.; Quinn, M. T.; Khlebnikov, A. I. и Schepetkin, I. A. (2009): Synthesis, characterization and potent superoxide dismutase-like activity of novel bis(pyrazole)-2,2-bipyridyl mixed ligand copper(II) complexes. *Complexes // Dalton Trans.*; 4488-4498.
14. Ritva, P. E.; Mandy, M. R.; Emily, H. and Carolyn, B. (1983): Pyrazole Effects on Mutagenicity and Toxicity of Dimethylnitrosamine in Wistar Rats. *Can. Res.*, 43: 496.
15. Wilson, W.L. and Bottiglieri, N.G. (1962): Phase I studies with pyrazole. *Can. Chem.*, 21: 137-141.
16. Yamada, M.; Murawaki, Y. and Hirayama, C. (1987): Effects of ethanol feeding on collagen synthesizing and degrading enzymes in rat pancreas. *Biochem. Pharmacol.*, (36) 20: 3361-3364.
17. Yongke, Lu.; Xiaodong, W. and Cederbaum, A. I. (2005): Lipopolysaccharide-induced liver injury in rats treated with the CYP2E1 inducer pyrazole. *J. Physiol. Gastro. and Liver Physiol.*, (289)308.