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Effect of 1,5-Bis (3,5-dimethylpyrazol-1-yl)-3-oxapentane Diacetatocopper on Kidney and Spleen of Rats: Histological and Immunohistochemical Evaluation

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Authors' contributions

This work was carried out in collaboration between all authors. Author AEN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author RAA managed the analyses of the study. Author FAEM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The present work was carried out to evaluate the histological and immunohistochemical changes of the kidney and spleen of rats after repeated exposure to 1,5-Bis (3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper (BDO). Two groups of male albino rats were used. The first group is the control and the second group was i.p. given 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper (12 mg / kg body weight) daily for 6 weeks. The results showed that the administration of BDO to rats exhibits various histological effects on tissues of kidney and spleen. Kidney from treated animals showed congestion of blood vessels, degeneration of renal tubules and glomeruli. The spleen tissue lost its characteristic structure and showing interference \ overlap between red and white pulp, enlarged trabecula and degenerated lymphocytes. Histochemical results revealed depletion of total proteins contents in renal tubules and glomeruli and spleen cells

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of treated rats. Expression of Bcl-2 protein was increased in the kidney and spleen of treated rats. It is concluded from the present work that BDO induced histopathological alterations and apoptosis in kidney and spleen of rats.

Keywords: Dimethylpyrazol; histology; Bcl-2 protein; kidney; spleen; rats.

1. INTRODUCTION

The search for new anti-diabetic drugs is one of the most important problems of modern medicine. A lot of pyrazole derivatives exhibit various pharmacological properties and are characterized by an antioxidant, antiviral activity, anti-inflammatory, immunomodulating properties, as well as antidepressive and anti-tumor effect [1]. It is also known that complexes of copper (II) with pyrazole derivatives exhibit biological activity as regulators of reactive oxygen species and superoxide dismutase [2]. The action 3,5-dimethylpyrazole is similar to that of insulin in intact rats [3].

1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper ($C_{18}H_{28}N_4O_5$-Cu) is one of the new derivatives and the most representative of pyrazole-containing copper (II) complexes, which was synthesized in the Polzunov Altai State Technical University (AltSTU) in 2007 year [4]. 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper is a hypoglycemic agent which decreased the level of glucose in the blood and are used in the treatment of diabetes mellitus [5].

The administration of antilipolytic drug (3,5-dimethylpyrazole at dose 12 mg/kg body weight) to rats affected both lipid and glucose metabolism of the animal in a very short time, this drug caused a dramatic and prolonged decrease in the plasma levels of free fatty acids and significantly lower glucose plasma levels [6]. The present work studied the effect of 1.5-Bis (3.5-Dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper on kidney and spleen of rats.

2. MATERIALS AND METHODS

2.1 1.5-Bis (3.5-Dimethylpyrazol-1-yl)-3-oxapentane Diacetatocopper Chemical Used

1.5-Bis(3.5-Dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper was dissolved in 0.9% physiological saline solution and injected intraperitoneally at dose 12 mg/kg body weight /daily for 6 weeks [3].

2.2 Animals and Treatments

In this study, 32 adult male albino rats (*Rattus norvegicus*) weighing 130 ± 5 g were used. Rats were breed in the Animal House of the Department of Zoology / Faculty of Science, Menoufia University) and placed under constant temperature (24 ± 2°C) throughout the experimental work. They were maintained on a standard rodent diet composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminized starch. Water was available *ad libitum*. Maintenance of animals and experimental procedures was approved by the animal ethical committee in accordance with the guide for care and use of laboratory animals (Approval No. MNFSH118). Animals were divided into 2 groups:

First group: animals of this group served as a control group and were injected intraperitoneally with saline solution.

Second group: animals of this group were injected intraperitoneally 1.5-Bis(3.5-Dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper dissolved in saline solution at a dose level of 12 mg/kg body weight /daily for 6 weeks.

2.3 Histological Study

For histological study, animals were sacrificed after 6 weeks; kidney and spleen were immediately removed and fixed in 10% neutral formalin for 24 hours**.** After fixation, specimens were dehydrated in ascending series of ethyl alcohol, cleared into two changes of xylene, infiltrated in three changes of molten paraffin wax with melting point of 58- 60°C and then embedded in molten paraffin blocks. Sections of 5 microns thickness were cut by using rotary microtome and mounted on clean slides. For histological examination, sections stained with Ehrlich's hematoxylin and counter stained with Eosin [7].

2.4 Histochemical Procedures

For histochemical purposes, small pieces of kidney and spleen 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 [8].

2.5 Immunohistochemical Study

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) [9].

The sections were deparaffinized and rehydrated routinely. Antigens were retrieved by heating the sections in a microwave oven at 700 W in 10 mmol/L citrate buffer (pH 6.0) for 10 min. After blocked with 3 mL/L H_2O_2 and swine serum, specimens were then incubated with the primary antibodies, directed against Bcl-2. The staining was performed by streptavidin peroxidase enzyme conjugate method using a S-P kit (Zymed). Reaction products were visualized by DAB. Brown-yellow granules in cytoplasm were recognized as positive staining [9].

2.6 Image Analysis

Digital images were analyzed by a semi-quantitative scoring system (Image J software, Java based application for analyzing images). The immune-stained sections were analyzed in 10 microscopic fields under high-power field (×400) microscope. In each field, the immunopositive (brown) area was recorded. Percentage of positive stained area (%) was calculated as mean of 10 fields / slide.

2.7 Statistical Analysis

Data were expressed as mean ± standard error (SE). The significance of differences means evaluated by using independent sample t-test. Statistical program of social sciences (SPSS) software for windows, version 20 was used.

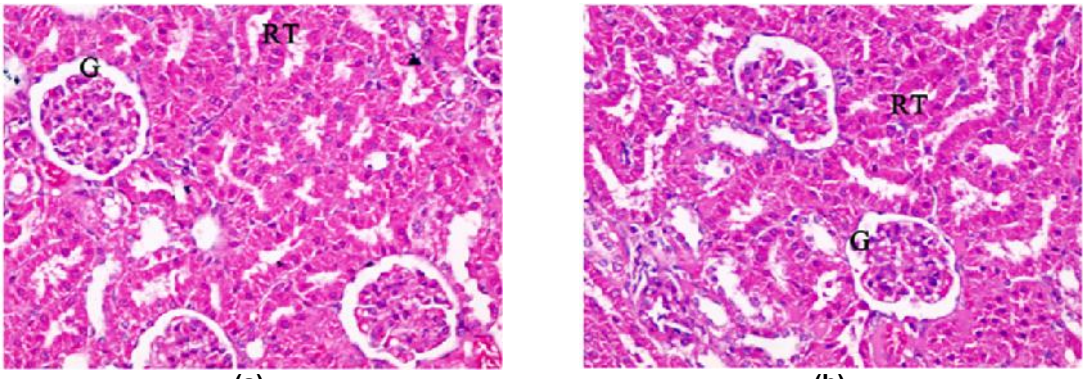
3. RESULTS

3.1 Histological Observation

3.1.1 Kidney

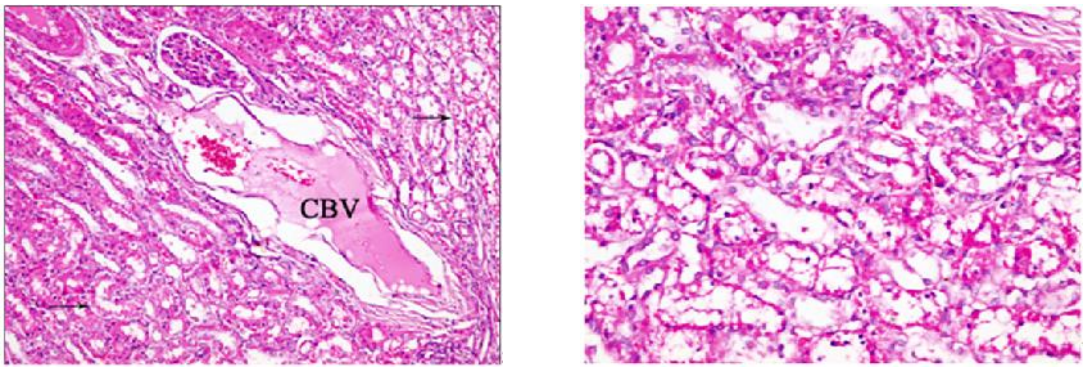
Examination of the kidney sections of control rat after 2, 4 and 6 weeks revealed normal histological structures of the renal cortex which consists of renal corpuscles, proximal and distal convoluted tubules. Renal corpuscles consist of Bowman'capsule is enclosed with a tuft of glumerular capillaries and double membrane with urinary space inbetween (Fig.1).

Kidney sections of rats treated with BDO for 2 weeks showed degeneration and deterioration of the cortical constituents with damaged tubule, as well as, enlarged and congested renal veins (Fig. 2). The Malpighian corpuscles after 4 weeks of treatment lost their characteristic configuration with atrophied and fragmented glomerulus, moreover, the epithelial cells lining renal tubules were exfoliated from their underlying basement membrane and degenerated with vacuolated cytoplasm and pyknotic nuclei, as well as, enlarged and congested renal veins was observed (Fig. 3). While, after 6 weeks, a number of glomerular capillaries were suffering from severe signs of glomerular congestion, atrophy, fragmentation and degeneration, while others were lost their attachments and mesangial stroma. Congestion and dilatation of blood vessel were observed, moreover, the renal tubules suffering from degeneration and their lumen were filled with proteinaceous casts (Fig. 4).



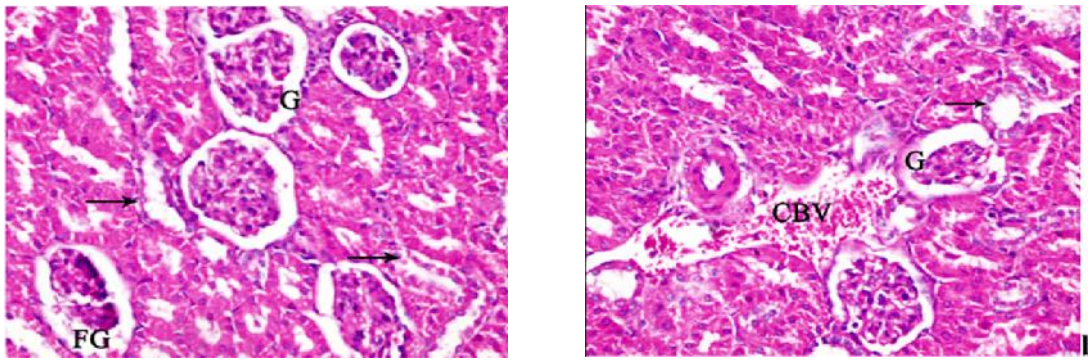
(a) (b)

Fig. 1(a,b). Sections of control kidney rats showing the glomerulus (G) and renal tubules (RT), X 400



(a) (b)

Fig. 2(a,b). Kidney of rats after treatment with 1.5-Bis(3.5-Dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper for 2 weeks showing congested blood vessel (CBV), damaged tubule (arrow), X 400



(a) (b)

Fig. 3(a,b). Kidney of rats after 4 weeks treatment with BDO showing exfoliation of epithelial cells of some renal tubules with vacuolated cytoplasm and pyknotic nuclei (arrow), atrophied glomerulus (G), Fragmented glomerulus (FG), congested blood vessel (CBV), X 400

3.1.2 Spleen

Examination of the spleen sections of control rat after 2, 4 and 6 weeks revealed normal histological structures. The normal spleen in the control group displayed splenic parenchyma composed of white and red pulp surrounded by a capsule of dense connective tissue, from which emanated trabeculae dividing the splenic parenchyma into incomplete compartments (Fig. 5). The spleen sections of animals treated with BDO for 2 weeks showed low lymphocyte densities, more fibroblasts, more trabeculae and the lymphocytes appeared with pyknotic nuclei (Fig. 6). After 4 weeks of treatment, the spleen tissue lost its characteristic structure and showing interference \ overlap between red and white pulp, enlarged trabecula (Fig. 7). These changes were more apparent in spleen sections examined after 6 weeks. In these specimens, congestion of splenic cords and sinuses and dilated congested blood vessel were observed. The trabeculae were enlarged and most of the lymphocytes were damged with pyknotic nuclei (Fig. 8).

3.2 Histochemical Results

In control kidney of rat, the protein materials are positively stained in the form of small bluish irregular particles in cytoplasm of the cells of renal tubules, the nuclear envelope, chromatin materials and nucleoli (Fig. 9). Examination of kidney of rats after 2, 4, 6 weeks of treatment with BDO showed a gradual reduction of protein content of the cells lining tubular epithelia and the glomeruli (Fig. 9).

The protein materials of spleen of control rat are positively stained in the form of small bluish irregular particles in cytoplasm of all the cells, the nuclear envelope, chromatin materials and nucleoli (Fig. 10). Examination of spleen of rats after 2, 4, 6 weeks of treatment with BDO showed a reduction of protein content of all cells (Fig. 10).

3.3 Immunohistochemical Results

Normal kidney rat demonstrated Bcl-2 immunopositivity primarily within the cytoplasm cells of distal tubule and rarely staining of the proximal tubular (Fig. 11). In contrast, Bcl-2 protein expression was increased in cytoplasm cells of renal tubules of kidney rats after 2, 4, 6 weeks of treatment with BDO (Fig. 11).

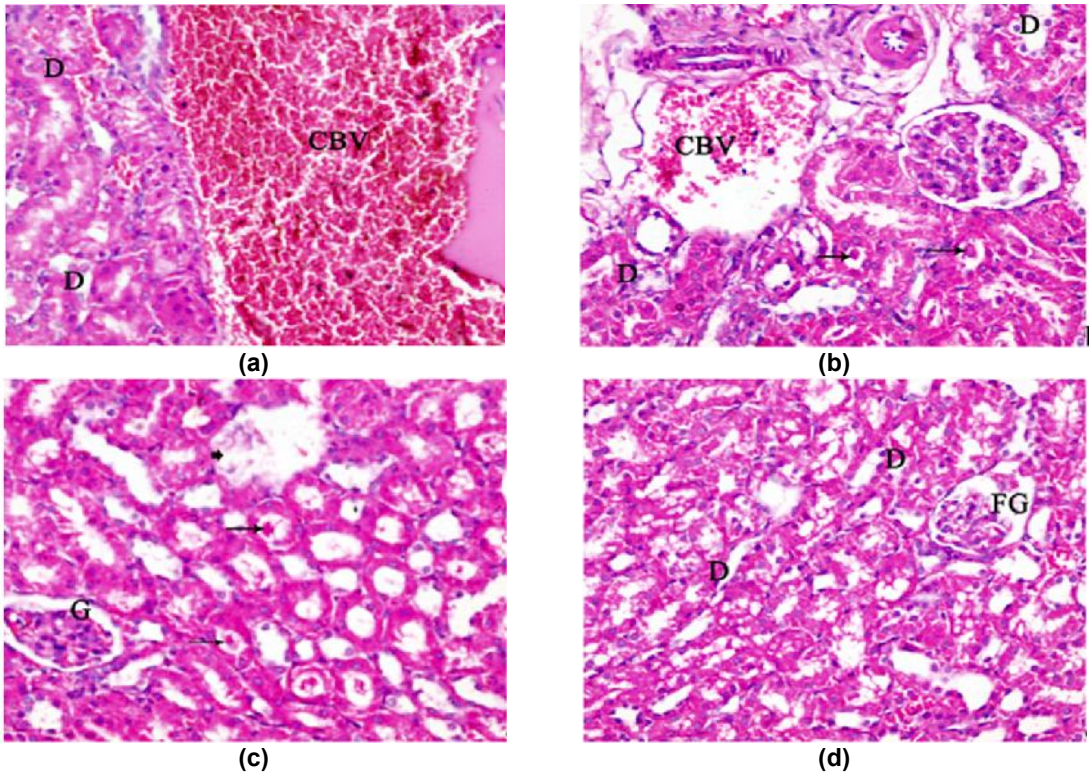


Fig. 4(a,b,c,d). Kidney of rat after 6 weeks treatment with BDO showing congested blood vessel (CBV), damaged tubule (D), atrophied degenerated glomeruli (G), proteinaceous casts in the lumen of the renal tubules (arrow), edema (arrow), Fragmented glomerulus (FG), X 400

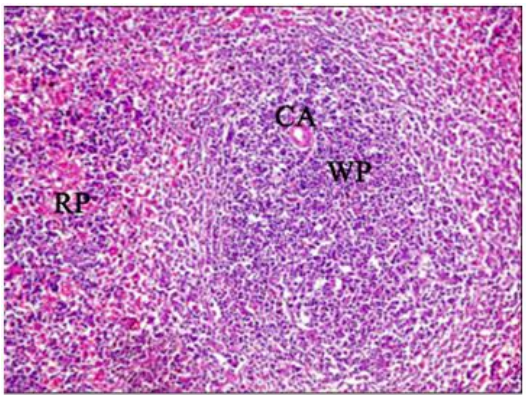
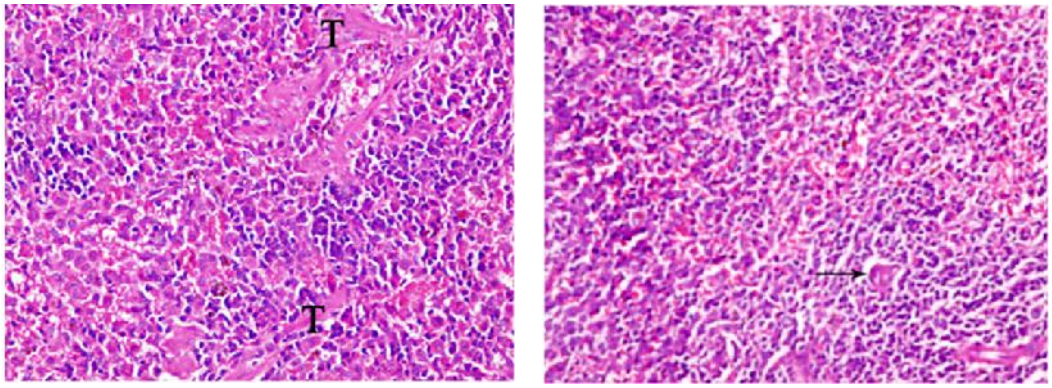
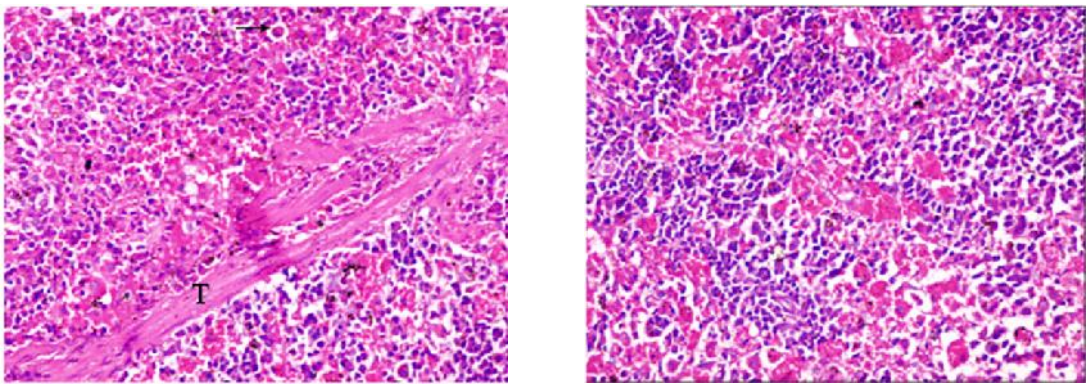


Fig. 5. Spleen of a control rat showing red pulp (RP), white pulp (WP) and central arteriole (CA), X 400



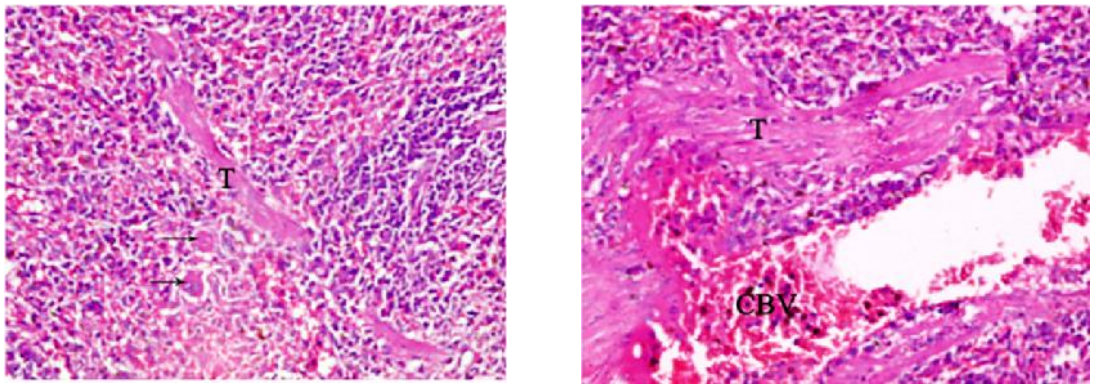
(a) (b)

Fig. 6(a,b). Spleen of rat after treatment with 1.5-Bis(3.5-Dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper for 2 weeks showing fibroblasts, large bundles of fibers of trabecula (T) and pyknotic nuclei (arrow), X 400



(a) (b)

Fig. 7(a,b). Spleen of rat after 4 weeks treatment with BDO showing interference between red and white pulp, enlarged trabecula (T), X 400



(a) (b)

Fig. 8(a,b). Spleen of rat after 6 weeks treatment with BDO showing dilated congested blood vessel (CBV), enlarged trabecula (T), congestion of splenic cords and sinuses also, pyknotic nuclei (arrow), X 400

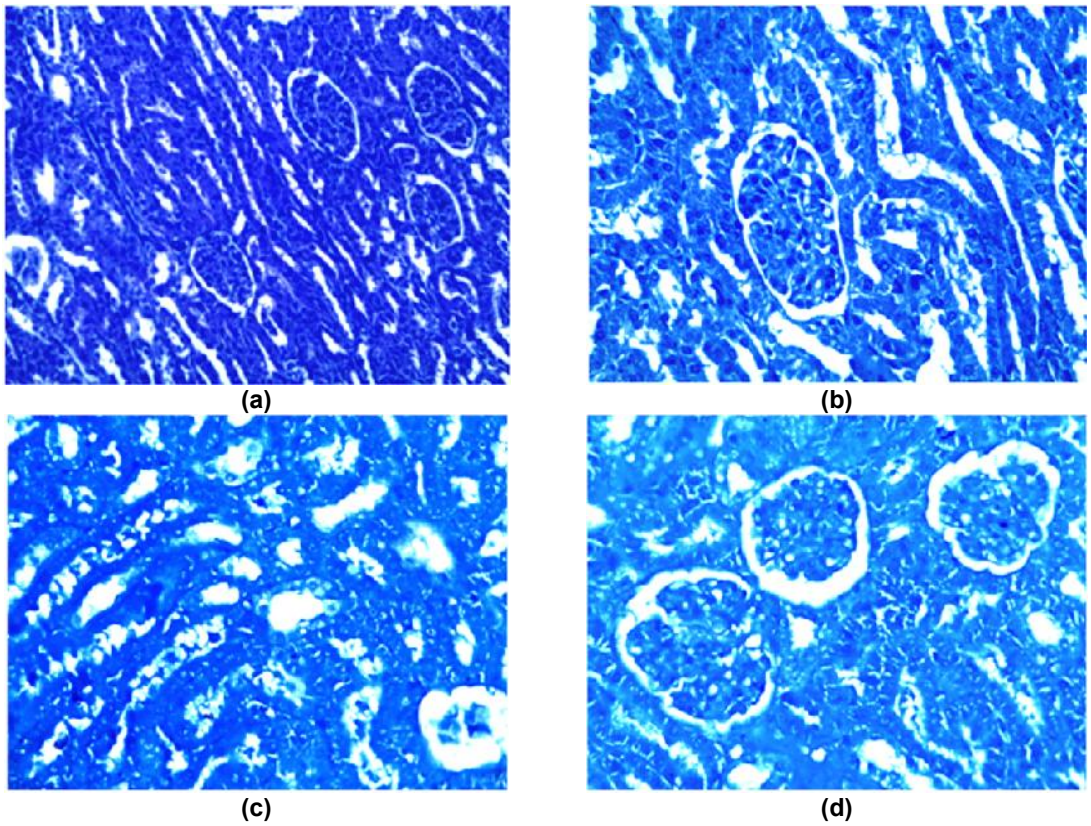


Fig. 9. Sections of Kidney showing (a) normal protein content in renal tubules and glomeruli of control rats, (b,c,d) marked reduction of total proteins in rats treated with 1.5-Bis(3.5-Dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper, X 400

Spleen of normal rat demonstrated rarely Bcl-2 immunopositivity staining (Fig. 12). In contrast, Bcl-2 protein expression was increased in spleen rats after 2, 4, 6 weeks of treatment with BDO (Fig. 12).

3.4 Image Analysis of Immunohisto-chemical Reaction of Bcl-2

Treating rats with BDO for 6 weeks resulted in a significant increase (p<0.01) in the Bcl-2 protein expression in spleen and kidney when compared with the normal control group (Table 1).

4. DISCUSSION

The results of the present investigation demonstrated the adverse effect of 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper on kidney and spleen of rat. Exposing rats to 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper for 6 weeks was found to induce different histopathological alterations. The kidney showed congestion of renal blood vessels, degeneration of renal tubules and glomeruli. The spleen appeared with congestion of splenic blood vessels, low lymphocyte densities, more fibroblasts, more trabeculae and degeneration of cellular components. Similarly, Nofal [10] reported that liver sections after treatment with the same drug (BDO) manifested loss of normal hepatic structure, inflammatory infiltration, marked enlarged vacuolated cytoplasm in hepatic cells, congestion of blood vessels.

These alterations seemed to follow almost the same pattern as that previously enumerated by some investigators under the effect of different derivatives of pyrazole. Wilson and Bottiglieri [11] reported that pyrazole induced primary toxicity in the liver, kidney and bone marrow of human. Intraperitoneal injection of pyrazole enhanced liver injury, oxidative stress, induction of apoptosis and necrosis [12]. Pyrazole induced oxidative liver injury in mice [13]. The administration of 3,5-dimethylpyrazole at dose 12 mg/kg body weight revealed many vacuolated

lysosomes and autophagic vacuoles in the liver cells of rats [6].

Histochemical results revealed depletion of total proteins contents in kidney and spleen of rats treated with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper for 6 weeks. Also, the administration of 3,5-dimethylpyrazole at dose 12 mg/kg body weight caused intracellular degradation of proteins in rat liver cells [6]. The administration of rat with 3,5dimethylpyrazole at dose 12 mg/Kg body weight decreased significantly the peroxisomal enzyme activities and increased degradation of liver proteins [14]. Lindros [15] reported that chronic treatment rats with the higher dose of 4-methylpyrazole caused a relative decrease in the liver protein content.

Apoptosis is an essential physiological process for the selective elimination of cells, which is involved in a variety of biological events. The Bcl-2 family is the protein family involved in the regulation of apoptotic cell death. Bcl2 is anti-

Table 1. The mean percentage area of Bcl-2 positive staining in kidney and spleen of rats in control and BDO groups

Animal groups	Spleen	Kidney
Control	0.592± 0.008	2.712± 0.005
2 weeks	2.721± 0.005*	12.998± 0.009*
4 weeks	5.822± 0.008**	20.118± 0.01**
6 weeks	32.518± 0.01**	37.051± 0.004**

Data presented as means ± standard errors
(*) *Significant difference as compared with control group at P < 0.05*
(**) *Significant difference as compared with control group at P < 0.01*

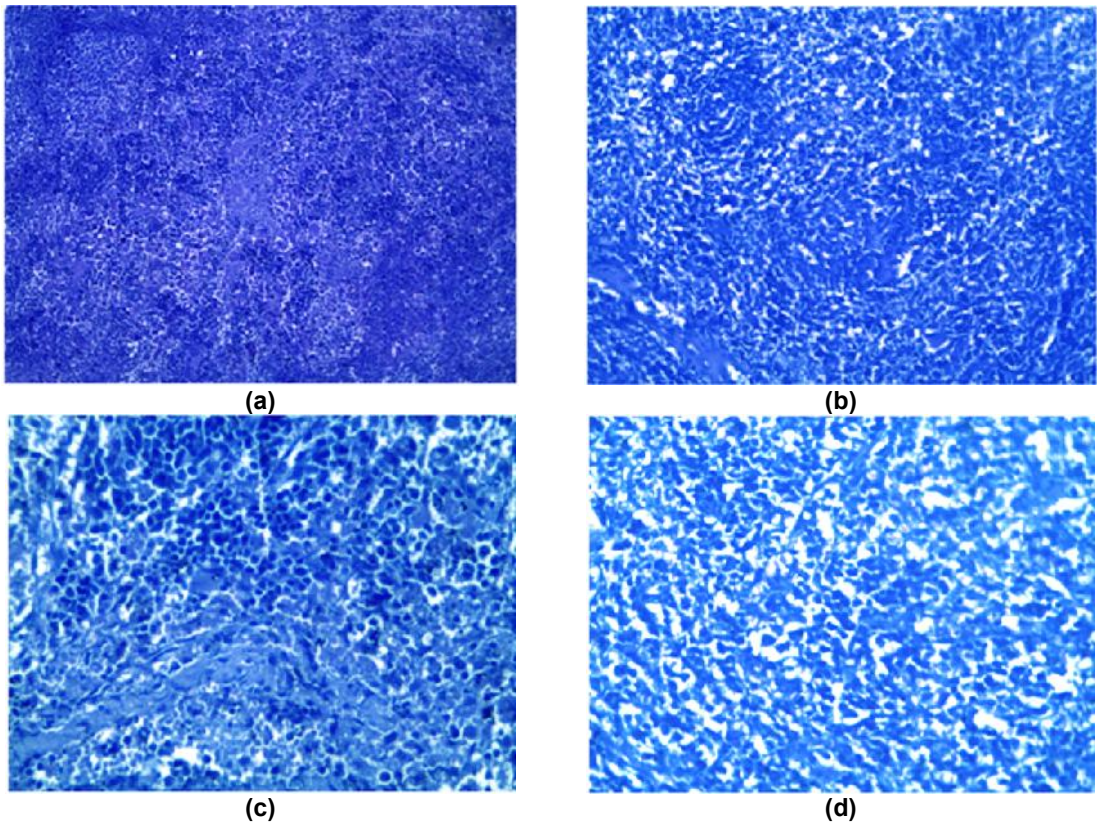


Fig. 10. Sections of spleen showing (a) normal protein content in control rats,(b,c,d) marked reduction of total proteins in rats treated with 1.5-Bis(3.5-Dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper, X 400

apoptotic members prevent apoptosis either by sequestering proforms of death-driving cysteine proteases called caspases (a complex called the apoptosome) or by preventing the release of mitochondrial apoptogenic factors such as cytochrome c and AIF (apoptosis-inducing factor) into the cytoplasm. After entering the cytoplasm, cytochrome c and AIF directly activate caspases that cleave a set of cellular proteins to cause apoptotic changes [16]. In the present work, treating rats with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper for 6 weeks caused marked expression of Bcl-2 in the kidney and spleen cells. In agreement with this result, Nofal [10] revealed increase expression of Bcl-2 proteins in hepatic cells of rats treated with the BDO. Bcl-2 protein was exclusively positive expressed by ductular epithelial cells [17]. Bcl-2 protein is over expressed in the majority of renal cell carcinomas and plays a role in tumorigenesis [18].

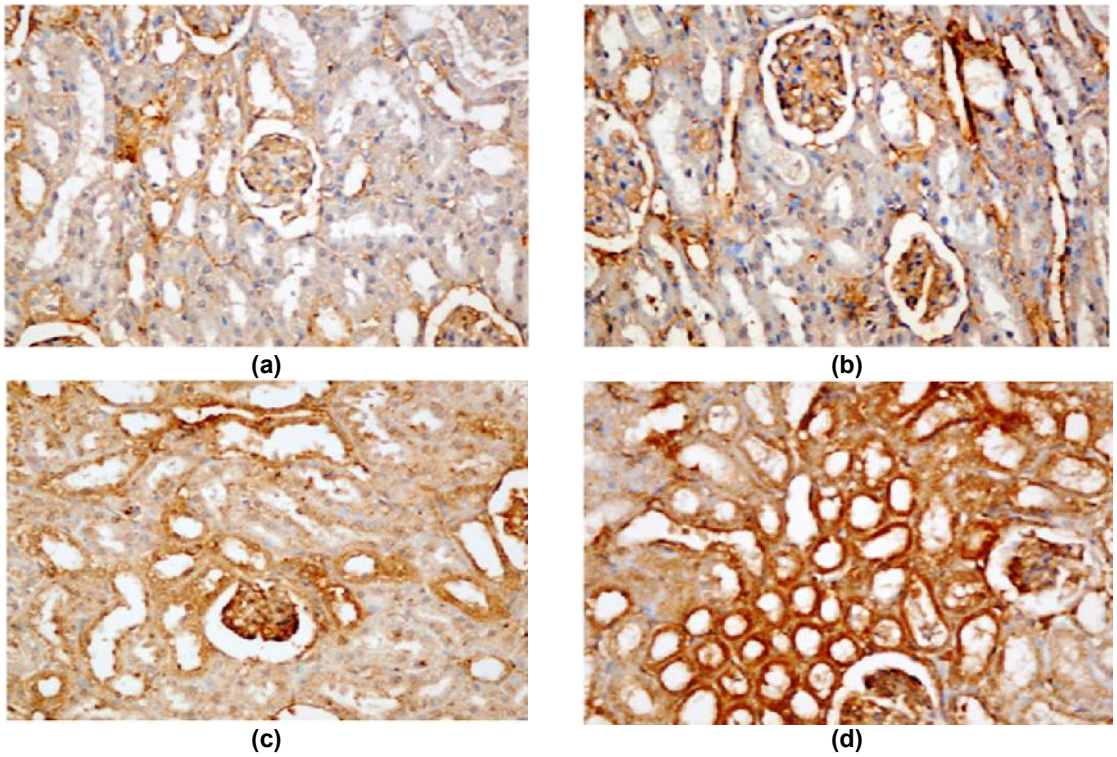
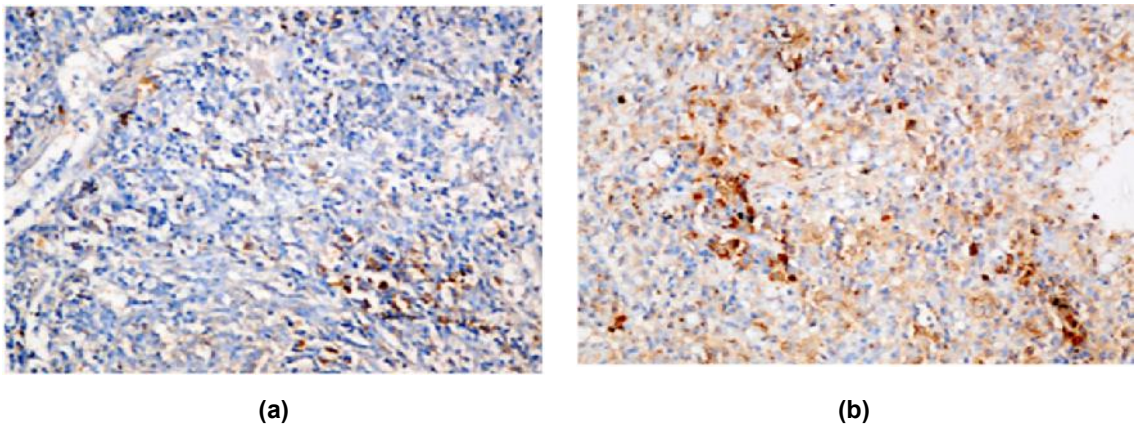
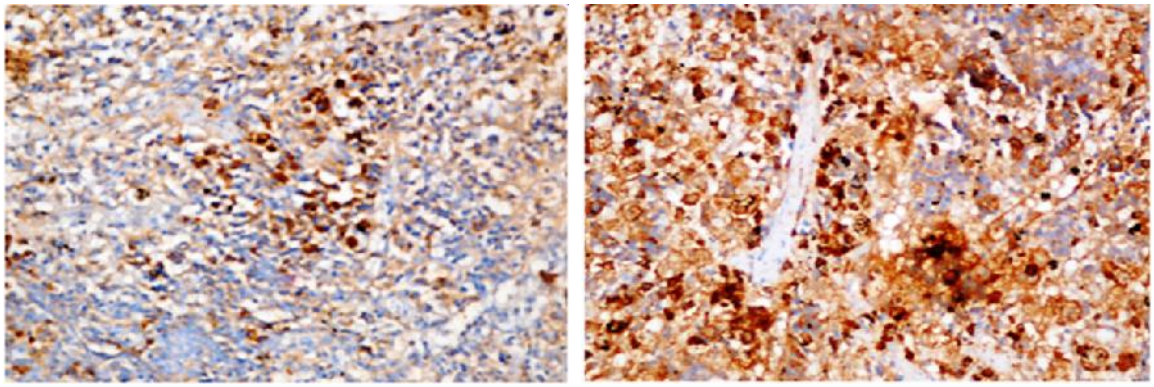


Fig. 11. Kidney sections of (a) control rats showing few expression of Bcl-2 protein, (b,c,d) rats treated with 1.5-Bis(3.5-Dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper showing increase in expression of Bcl-2 protein (Immunostain, X 400).





(C) (d)

Fig. 12. Spleen sections of (a) control rats showing few expression of Bcl-2 protein, (b,c,d) increase in expression of Bcl-2 in rats treated with 1.5-Bis(3.5-Dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper (Immunostain, X 400).

5. CONCLUSION

It is concluded from the present work that BDO induced histopathological alterations and apoptosis in kidney and spleen of rats.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Choia MK, Hana JK, Kima HJ. Aqueous extract of *Artemisia capillaris* exerts hepatoprotective action in alcohol-pyrazole-fed rat model. J. Ethnopharmacol. 2013;147:662-670.
2. Potapov AS, Nudnova EA, Domina GA, Kirpotina LN, Quinn MT, Khlebnikov AI, Schepetkin IA. Synthesis, characterization and potent superoxide dismutase-like activity of novel bis(pyrazole)–2,2-bipyridyl mixed ligand copper(II) complexes. Complexes,Dalton Trans. 2009;4488-4498.
3. Cavallini G, Donatia A, Bergamini E. Antiaging therapy: A novel target for antilipolytic drugs. Mini. Rev. Med. Chem. 2014;14(7):551-556.
4. Potapov AS, Domina GA, Domina GA, Khlebnikov AI, Ogorodnikov VD. Facile synthesis of flexible Bis(pyrazol-1-yl) alkane and related ligands in a superbasic medium. Eur. J. Org. Chem. 2007;5112-5116.
5. Nofal AE, Lampatov VV, Lepilov AV. Pancreatic response after treatment with 1,5-Bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper in rats. Journal of Cell Biology and Genetics. 2015;1(5):1-9.
6. Bergamini E, Tata VDE, Loccr Cubeddu T, Masiello P, Pollera M. Increased degradation in rat liver induced by antilipolytic agents: A model for studying autophagy and protein degradation in Liver. Exp. and Mol. Pathol. 1987;46:114-122.
7. Lillie RD, Fulmer HM. Histopathological technique and practical histochemistry. 4th edn. New York, Mc Graw Hill. 1976;7.
8. Pearse AGE. Histochemistry, theoretical and applied. 3rd end. Churchill Livingstone. London. 1972;2.
9. Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford K, Stein H, Mason Dy. Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J. Histochem. Cytochem. 1984;32:219-229.
10. Nofal AE, Potapov AS, Khlebnikov AI. Immunohistochemical detection of Bcl-2

Protein in normal and abnormal rat Liver. International Journal of Advanced Research in Chemical Science (IJARCS). 2014;1(1):1–8.
11. Wilson WL, Bottiglieri NG. Phase I studies with pyrazole. Can. Chem. 1962;21:137-141.
12. Lu Y, Cederbaum AI. Enhancement by pyrazole of lipopolysaccharide-induced liver injury in mice: role of cytochrome P^{450} 2E1 and 2A5. Hepatol. 2006;(44)1:263-274.
13. Bae SH, Sung SH, Lee HE, Kang HT, Lee SK, Oh SY, Woo HA, Kil IS, Rhee SG. Peroxiredoxin III and sulfiredoxin together protect mice from pyrazole-induced oxidative liver injury. Antioxid. Redox. Signal. 2012;(17)10:1351-1361.
14. Bergamini E, Segal HL. Effects of antilipolytic drugs on hepatic peroxisomes. Biol. and Med. 1987;295-303.
15. Lindros Ko, Stowell L, Vananen H, Siponen P, Laminsivu U, Pikarainen P, Salaspuro M. Uninterrupted prolonged ethanol oxidation as a main pathogenetic factor of alcoholic liver damage: Evidence from a new liquid diet animal model. Liver. 1983;3:79-91.
16. Tsujimoto Y. Role of Bcl-2 family proteins in apoptosis: Apoptosomes or mitochondria?. Genes Cells. 1998;3(11): 697-707.
17. Frederic C, Aurore L, Nadine M, Yvette G, Martin N, Phillppe G, Elie SZ. Immunohistochemical Detection of bcl-2 Protein in normal and pathological human liver. Am. J. of Pathol. 1994;(144)3:460-465.
18. Andrew H, Patricia DF, Regina GE, Ralph W, Devere W, Roger KL. Immunohistochemical Analysis of Bcl-2 Protein Expression In Renal Cell Carcinoma. American Urological Association (AUA) Journals. 1999;162(2): 610–613.

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