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Research Article

BIO CHEMICAL EFFECT OF 1, 5-BIS (3, 5-DIMETHYLPYRAZOL-1-YL)-3-OXAPENTANE-DIACETATOCOPPER IN ALBINO RATS

Amany Nofal[1, 2], Andrei Pota ov[1], Andrei Khlebnikov[1]

[1]*I. Polzunov Altai State Technical University, Barnaul, Russia*

[2]*Zoology department, Faculty of science, Menoufia University, Egypt*

ABSTRACT

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. It affected blood and liver biochemistry in rats. 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, while reported a significant increase in ALT and AST. Moreover, significant decrease in body weight.

Keywords: 1, 5-Bis (3, 5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper, ALT, AST, glucose and albumin.

INTRODUCTION

Diabetes mellitus is a disease leads to complications including heart disease, kidney failure and nerve damage. Insulin stimulated the uptake, storage and use of glucose by tissues. These activities result in a decrease in the level of blood glucose. Glucagon is hormone promotes the release of glycogen into the blood and thusincrease blood glucose levels (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 diabetesmellitus) 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 (Locci andBergamini, 1983). Bergamini etal.(1987) revealed that antilipolytic drugs (3,5-dimethyl-pyrazole at dose 12 mg/kg body weight) cause a dramatic and prolonged decrease in rat plasma levels of free fatty acids and significantly lower glucose plasma levels. as well as, a dramatic increase in the plasma glucagon ratio.

The administration of antilipolytic drugs (3,5-dimethylpyrazole at dose 12 mg/kg body weight) to rats can affect both lipid and glucose metabolism and the endocrine status of the animal in a very short time. In fact, these drugs cause a dramatic and prolonged decrease in the plasma levels of free fatty acids in 15 minutes and significantly lower glucose plasma levels (Bergamini et al.,1987).

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 (Donati et al., 2008).

Serum albumin is the most abundant blood plasma protein and produced only in the liver. Albumin is also very important in the transportation of many substances such as drugs, lipids, hormones, and toxins that are bound to albumin in the bloodstream. Serum albumin is generally used to assess the nutritional status, severity of disease, disease progression and prognosis (Gibbs et al., 1999). Low serum albumin has also been shown to be an independent indicator for prognosis in cancer patients with unknown primaries (Seve et al., 2006).

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 (Potapov et al.,2007).

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-diacetatocopper (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 (Locci et al., 1985) and (Donatia et al., 2008).

Experimental design:

The animals were divided into 3 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- Group1:

Animals of this group (25 rats) were injected intraperitoneally (ip) by 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper freshly dissolved in saline (12 mg/kg b.w.). Animals were sacrificed after

30min, 1.45hr, 2.30 hr and 24 hr of injection.

3- 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.

Change in body weight:

At the beginning of the experiment, the body weight of the animals were recorded and continuously recorded after 2, 4 and 6 weeks of treatment.

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 30min, 1.45hr, 2.30 hr, 24hr, 2, 4 and 6 weeks from starting the experiment.

a- Determination of alanine aminotransferase (ALT):

ALT was determined according to Gella et al. (1985) using Spinreact, S.A. Ctra. Santa Coloma, Spain kits.

b- Aspartate aminotransferase (AST):

AST was determined according to the methods of Gella et al. (1985) using Spinreact, S.A. Ctra. Santa Coloma, Spain kits.

c- Serum albumin:

Serum albumin was determined according the method of Doumas et al. (1971).

d- Serum glucose:

Serum glucose was determined according the method of Bergmeyer et al.(1974).

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

Change in body and testes weight:

Data exist in figure (1) showed that daily treatment with 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper for 2, 4 and 6 weeks caused significant decrease in body weight of rats in comparison with control group.

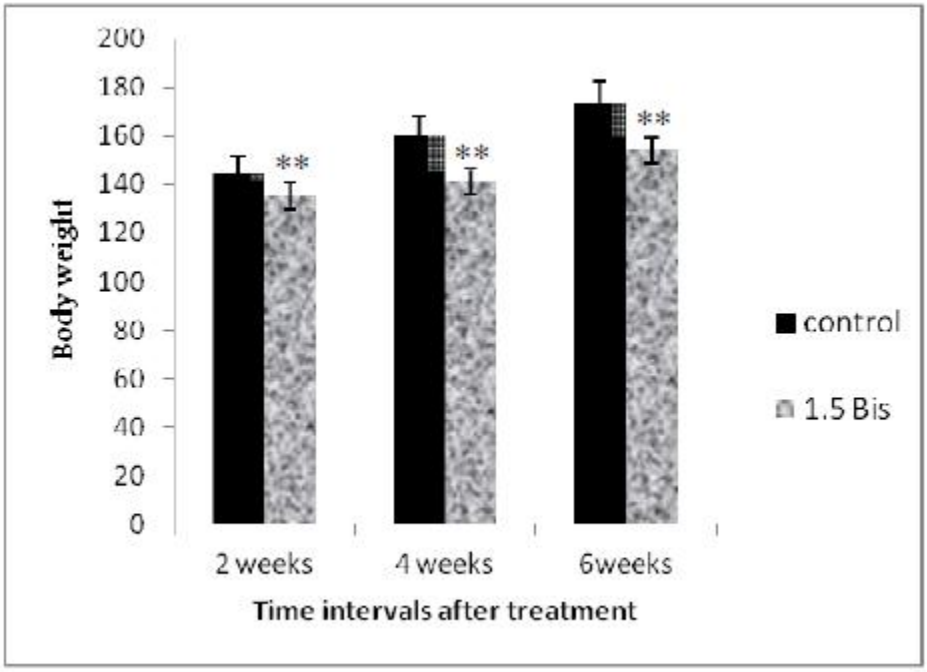


Figure 1: Effectofdifferenttreatmentsonbodyweight.

(**): highly significant at in comparison with control group.

Biochemical results:

1-Serum aspartate aminotransferase (AST) (U/L):

Single intraperitoneal administration of 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper to rats revealed that biochemical determination of AST after 30min, 1.45hr, 2.30 hr and 24hr of injection clearly exhibited fluctuation between an increase sometimes and decrease in other times (Fig. 2). While animals daily treated for 2, 4 and 6 weeks revealed significant increase in serum AST compared with

control group (Fig. 3).

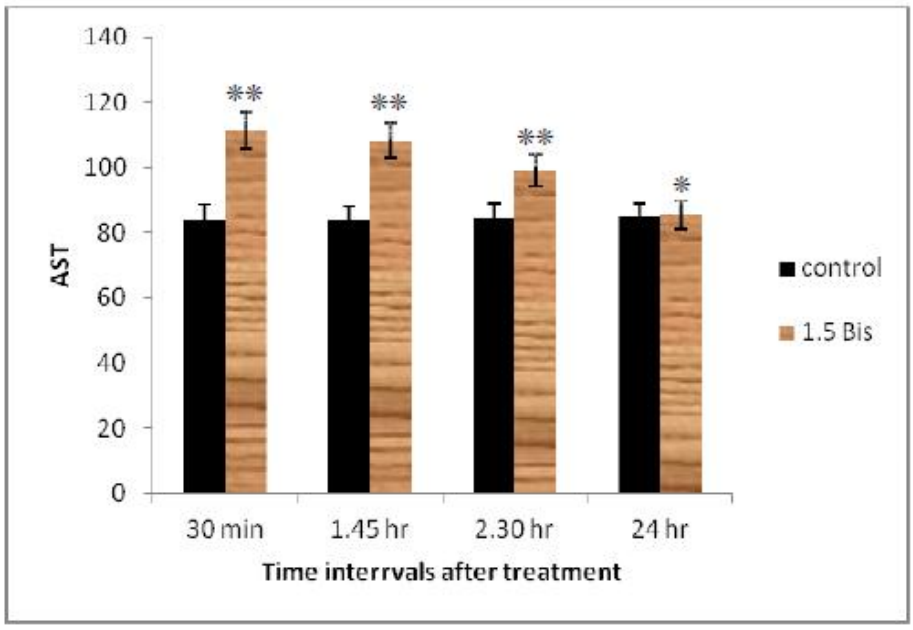


Figure 2: Effect of single intraperitoneal administration of 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper on serum level of AST (U/L). (*): low significant in comparison with control group,(**): highly significant in comparison with control group.

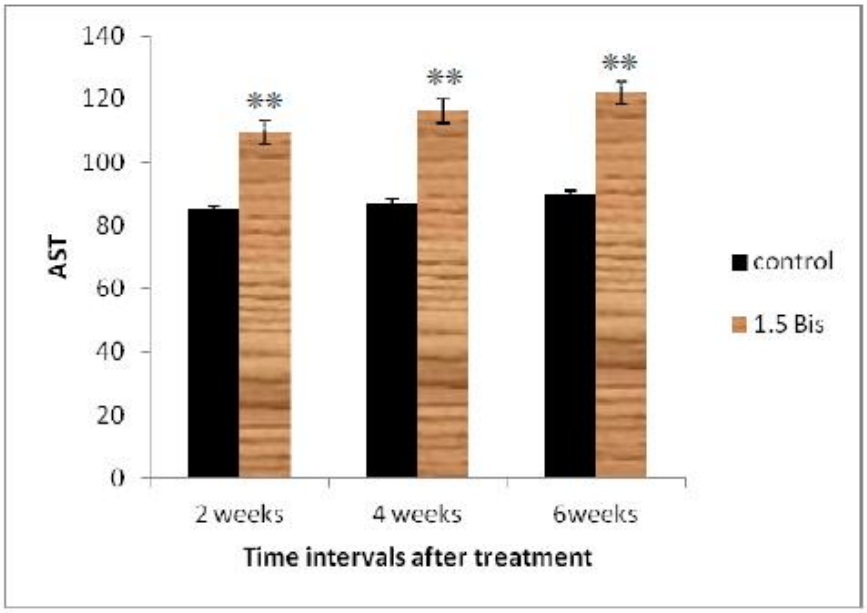


Figure 3: Effect of different treatments serum level of AST (U/L).(**): highly significant at in comparison with control group.

2-Serum alanine aminotransferase (ALT) (U/L):

Single intraperitoneal administration of 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper to rats revealed that biochemical determination of ALT after 30min, 1.45hr, 2.30 hr and 24hr of injection clearly exhibited fluctuation between an increase sometimes and decrease in other times (Fig. 4). While animalsdaily treated for 2, 4 and 6 weeks revealed significant increase in serum ALT compared with control group (Fig. 5).

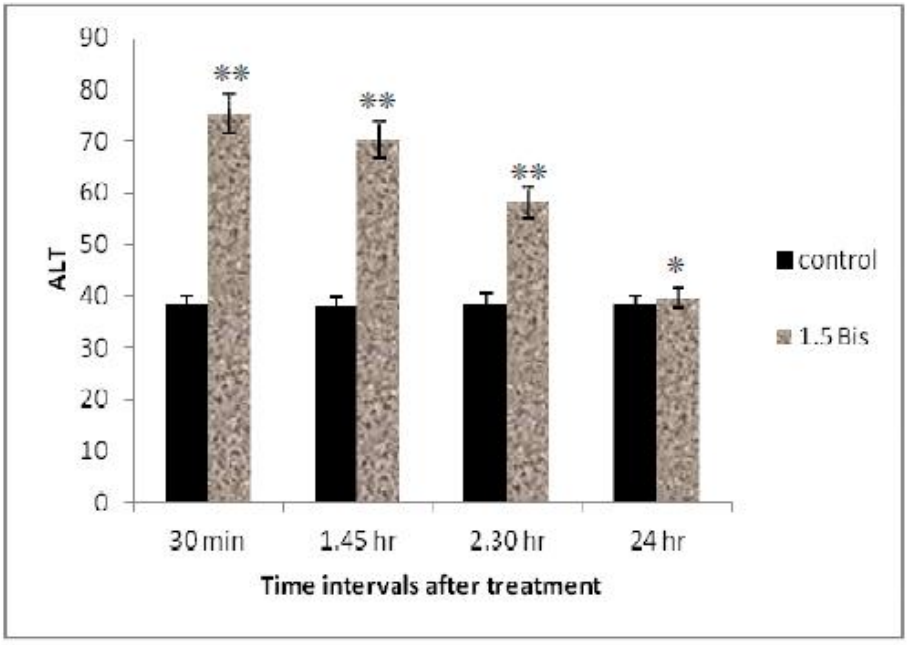


Figure 4: Effect of single intraperitoneal administration of 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper on serum level of ALT (U/L). (*): low significant in comparison with control group,(**): highly significant in comparison with control group.

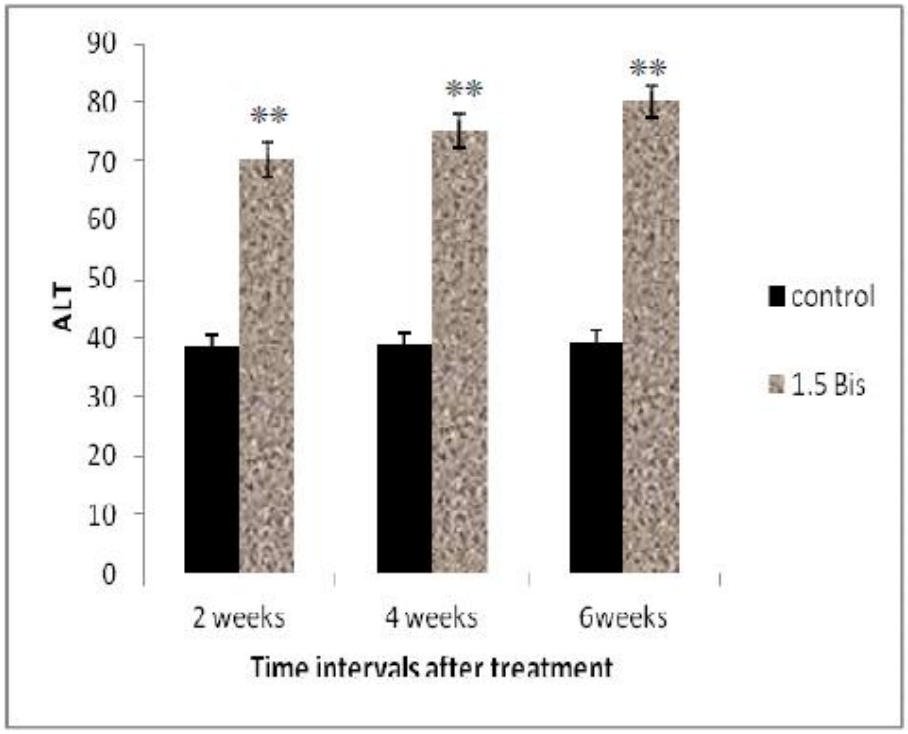


Figure 5: Effect of different treatments serum level of ALT (U/L).(**): highly significant at in comparison with control group.

3-Serum Albumin (g/dL):

Single intraperitoneal administration of 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper to rats revealed that biochemical determination of Albumin after 30min, 1.45hr, 2.30 hr and 24hr of injection clearly exhibited fluctuation between an increase sometimes and decrease in other times (Fig. 6). While animals daily treated for 2, 4 and 6 weeks revealed significant increase in serum Albumin compared with control group (Fig. 7).

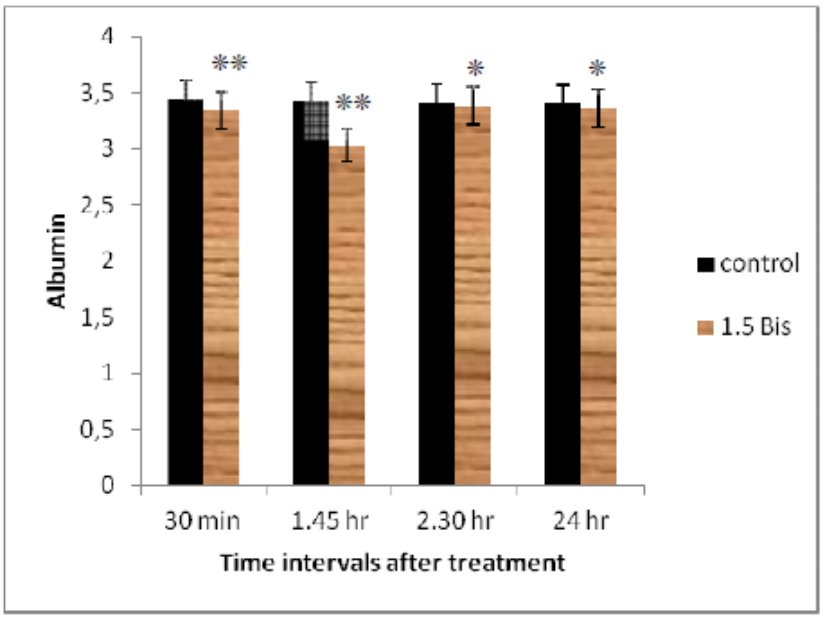


Figure 6: Effect of single intraperitoneal administration of 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper on serum level of albumin(g/dL). (*): low significant in comparison with control group,(**): highly significant in comparison with control group.

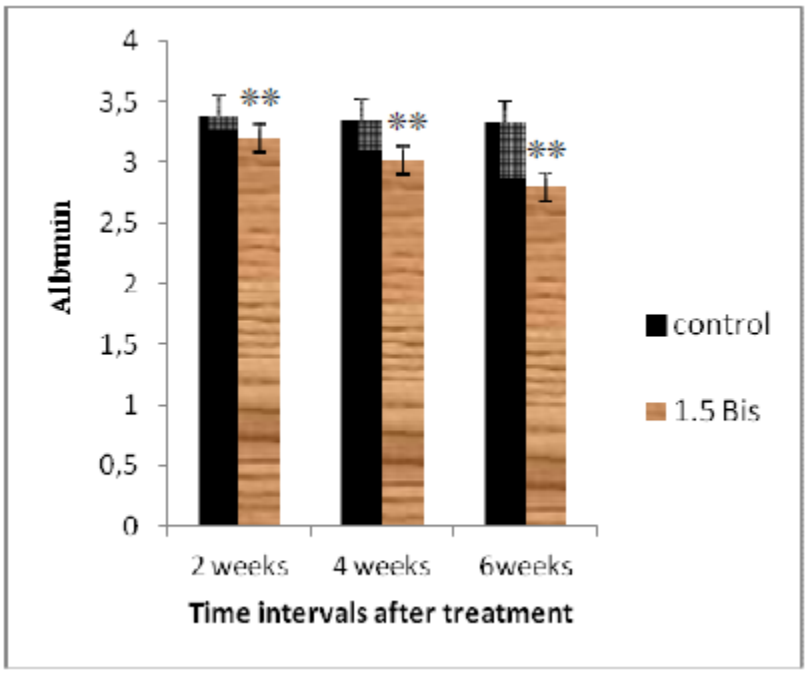


Figure 7: Effect of different treatments serum level of albumin (g/dL).(**): highly significant at in comparison with control group.

4-Serum glucose (mg/dl):

Single intraperitoneal administration of 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper to rats revealed that biochemical determination of serum glucose after 30min, 1.45hr, 2.30 hr and 24hr of injection clearly exhibited fluctuation between an increase sometimes and decrease in other times (Fig. 8). While animals daily treated for 2, 4 and 6 weeks revealed significant increase in serum glucose compared with control group (Fig. 9).

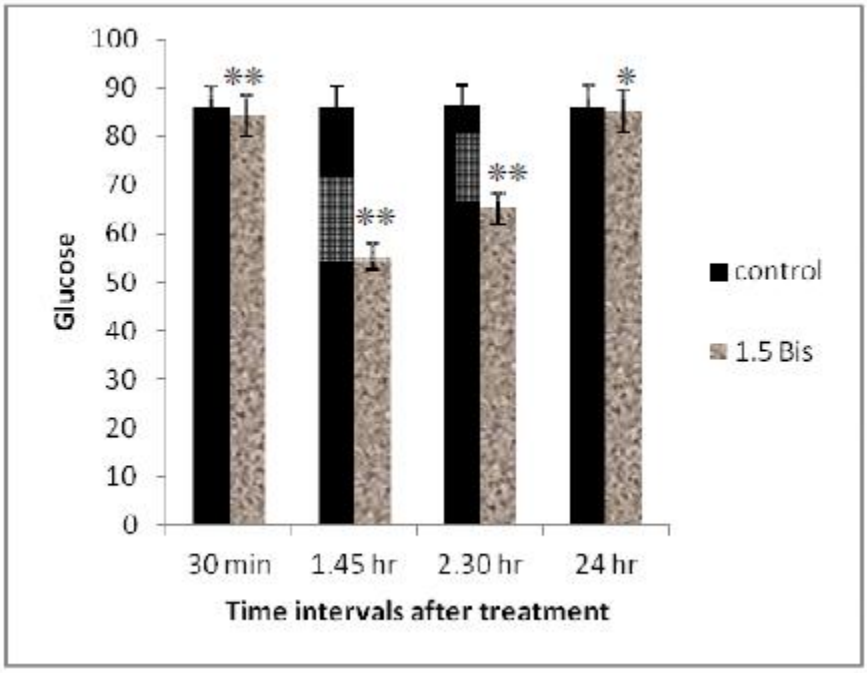


Figure 8: Effect of single intraperitoneal administration of 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper on serum level of glucose (mg/dL). (*): low significant in comparison with control group,(**): highly significant in comparison with control group.

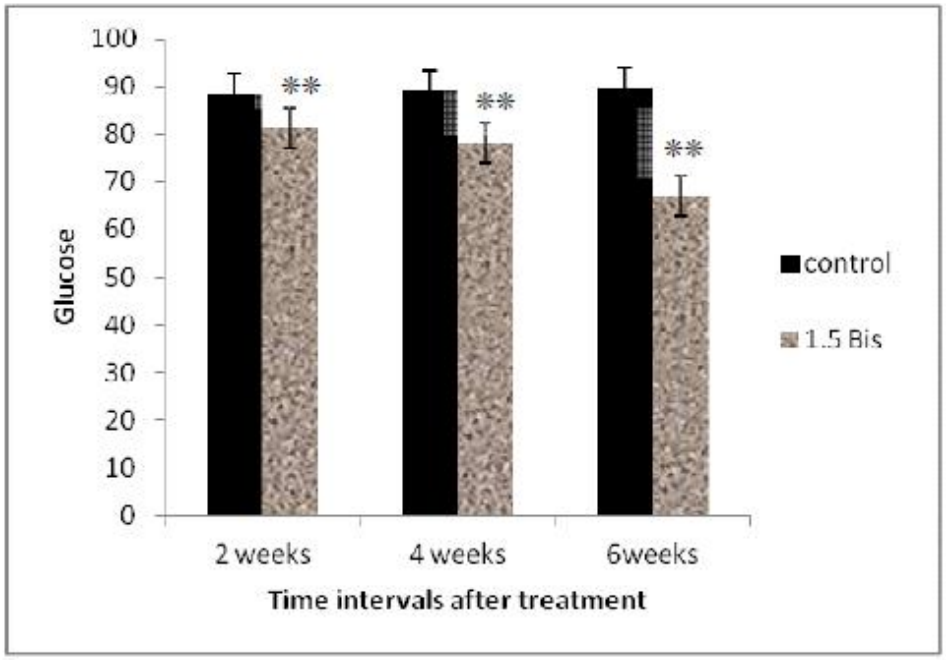
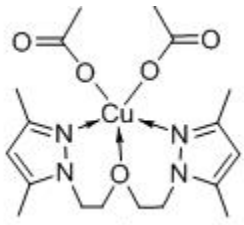


Figure 9: Effect of different treatments serum level of glucose (mg/dL).(**): highly significant at in comparison with control group.

DISCUSSION

Complex 1,5-bis(3,5-dimethylpyrazol-1-yl)-3-oxapentane diacetatocopper was prepared by the reaction of ligand and copper (11) acetate monohydrate in acetone solution similary to previously reported procedure for copper (11) nitrate complexes (Potapov et al., 2009). The proposed structure for this compound was confirmed by UV-Vis and IR spectroscopy, molar conductivity measurements and elementalanalysis data.



Our results indicated that treating rats with 1,5-Bis (3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper caused reduction in body weight, Such effect was time and age-dependent. These results were in agreement with, Locci et al. (1985), reported that treatment rats with antilipolytic drugs (3,5-

dimethylpyrazole at dose 12 mg/kg body weight) caused significant decrease in body and liver weight.

Examination of sera of animals after single intraperitoneal administration of 1,5-Bis(3,5-Dimethylpyrazol-1-yl)-3-oxapentane-diacetatocopper to rats revealed thatbiochemical determinations (ALT, AST, serum albumin and glucose) after 30min, 1.45hr, 2.30 hr and 24hr of injection clearly exhibited fluctuation between an increase sometimes and decrease in other times. Such effect was time and age-dependent. Donati et al., 2006 reported that the antilipolytic agent DMP (12 mg/Kg bw) was injected intraperitoneally to older rats 6 and 3hours before their sacrifice caused rapidly lowering free fatty acids (FFA), glucose and insulin plasma levels for almost 3hours.

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, while reported a significant increase in ALT and AST. Several investigators also obtained similar results. In this concern, Bergamini et al.,(1987), showed similar results of elevated activities of ALT and AST when rats administered with 3,5-dimethylpyrazole at dose 12 mg/kg body weight. The administration of antilipolytic drugs 3,5-dimethylpyrazole (DMP) to rats induced significant decrease in plasma FFA in 15 min, glucose and insulin (Donati et al.,2008).

Treatment of fasting rats with antilipolytic drugs (either 3,5-dimethyl-pyrazole 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. (Locci et al., 1985). The administration of antilipolytic drugs (3,5dimethylpyrazole at dose 12mg/Kg body weight) decreased the concentration of free fatty acids (FFA), 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 (Bergamini and Segal, 1987).

Treatment with 3,5-dimethylpyrazole at dose 12 mg/kg body weight resulted in a significant decrease in rat peroxisomal fatty acid-oxidative activity within a very short time (Locci and Bergamini, 1982 & 1983).

3,5-dimethylpyrazole administration at dose 12 mg/kg body weight every 3 hr causes an immediate and prolonged reduction in the free fatty acids plasma levels of rats. The fatty acid oxidative activity in the liver decreased very rapidly (changes are significant by hour 3) and reached its minimum by hour 5. Subsequently, the enzyme activity remained unchanged. 3,5-dimethylpyrazole was given to animals as a single injection . The effects of the drug on free fatty acids plasma levels were exhausted in3 hr. After this time, the free fatty acids plasma levels transiently increased above normal values. 4 hr after the restoration of free fatty acids plasma levels, the enzyme activity began to rise slowly towards normal values. Full restoration required 8 hr. within a few minutes of 3,5-dimethylpyrazole administration, immunoreactive

insulin decreased and immunoreactive glucagon plasma levels increased (Locci et al.,1985).

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).

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 (Lu and Cederbaum, 2006).

In conclusion, 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 in blood and liver biochemistry in rats.

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