MORPHOLOGY, PATHOLOGY, PHYSIOLOGY

Cite as: Archiv EuroMedica. 2022. 12; 5: e1. DOI 10.35630/2199-885X/2022/12/5.2

Received 13 July 2022;
Received in revised form 7 August 2022;
Published 9 September 2022

COMPARATIVE ANALYSIS OF TOXICITY OF BIS[BIS(3,5-DIMETHYLPYRAZOL-1-YL)ACETATO]COPPER AND CU_2(3,5-DIPS)$_4$(H_2O)$_3$ COPPER(II)-BASED COMPLEXES

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ABSTRACT

One of the priority tasks of modern medicine is the search for new effective means of facilitating the treatment of injuries of various nature. Besides the stability, ease of use and prolonged action, the requirements for such compounds also include low toxicity. In this regard, copper-based complexes with high antioxidant activity are of particular interest.

The aim of this work is to determine and compare the toxicity of bis[bis(3,5-dimethylpyrazol-1-yl)acetato]copper(II) complex, (Cu[HC(COO)(pz^{Me2})$_2$]$_2$ (complex **1**) and Cu_2(3,5-DIPS)$_4$(H_2O)$_3$ (complex **2**) by evaluating the survival, life expectancy and several blood parameters.

According to the obtained data, complexes **1** and **2** proved to be low-toxic compounds. According to the test results: in case of complex **1** at a dose of 1450 mg/kg, we received a survival rate of 87,5%, and an average life expectancy of 26,8 days, and in the case of complex **2** at the same dose, the survival rate was 75% and the average life expectancy was 22,9 days, which indicates the low toxicity of these compounds (complex **2** has however a higher toxicity, since an identical result is achieved with a lower dose). Using the method of integration according to Behrens in experiments on rats, an average lethal dose of $LD_{50/7}$ was found, which is more than 2000 mg/kg.

Regarding the blood parameters: in case of complex **1**, on the 3rd day of the experiment, an increase in the level of all studied blood parameters was observed. This was followed by a decrease, and on the 7th day there was a normalization of all blood parameters except for the number of platelets. Nevertheless, a trend towards normalization was observed in this indicator as well, which led to the normal value of platelet levels by the 21st day.

When complex **2** was administered subcutaneously, a significant decrease in hemoglobin level was observed, which was restored by the 7th day; a significant decrease in blood clotting time (BCT), which persisted until the end of the study. By the 7th day, a significant decrease in the levels of platelets and

erythrocytes was also noted.

The results of haematological studies also confirmed the lowest toxicity of complex **1**.

Keywords: copper(II) complexes, clotting time, number of erythrocytes, leukocytes, platelets, haemoglobin level, haematocrit.

INTRODUCTION

The purpose of toxicological studies of substances is to establish the nature and severity of their damaging effect on the body of experimental animals and to assess their safety.

Toxicological studies are conventionally subdivided into: studies of general toxic effects and studies of specific types of toxicity (allergenicity, immunotoxicity, reproductive toxicity, mutagenicity, carcinogenicity) [1].

The objectives of the study of general toxic action are:

- determination of tolerable and toxic doses of a substance;
- identification of the organs and systems of the body that are most sensitive to the studied substance, as well as the study of the reversibility of the damage caused;
- study of the dependence of toxic effects on the dose and duration of the use of the substance.

It is known that the hematopoietic system, as an actively proliferating tissue, is extremely sensitive to the action of various factors, including toxic components [2,3]. Therefore, the problem of the influence of pathogenic factors on the blood system can also be considered as characteristic of toxicity. Blood values can be used as indicators to detect toxic effects. It is very important to register the timing of the development of intoxication and the death of animals.

The aim of this work is to determine and compare the toxicity of bis[bis(3,5-dimethylpyrazol-1-yl)acetato]copper(II) complex (**1**) and Cu_2(3,5-DIPS)$_4$(H_2O)$_3$ (**2**) by determining survival, life expectancy and several blood parameters.

MATERIALS AND METHODS

A series of *in vivo* experiments was carried out on mature rats in compliance with current best practices and standards of care in laboratory animals. Animals of the same age were used, since the pharmacokinetics and, therefore, also the toxicity of the substance may change depending on age. The dispersion in the initial mass did not exceed ± 10%. It is also taken into account that the sensitivity of animals to a substance can change under the influence of a number of external factors (temperature, humidity, illumination, etc.).

Along with the experimental animals receiving the test substances, control animals should be kept in similar conditions.

The determination of the toxicity of the compounds was carried out in order to quantify the dose-effect relationship. Animals were divided into 3 groups (8 animals per each group): group I - with injection of complex **1**, II group - with injection of complex **2**, group III - control group - intact animals).

To determine the toxicity of Cu-based complexes Cu[HC(COO)(pz^{Me2})$_2$]$_2$ [4,5] and Cu_2(3,5-DIPS)$_4$(H_2O)$_3$, experiments were set on white, outbred, mature rats with an average weight of 185 g. Rats were injected subcutaneously with a solution (in DMSO) of these compounds in a volume of 2 mL.

The toxicity of metal complexes is usually characterized based on the calculation of the LD50/7 index, i.e. the dose of the compound at which the death of 50% of animals is observed within 7 days after subcutaneous administration of the substance into the body. To do this, metal complexes **1** and **2** are administered to animals in gradually increasing doses - from the most ineffective to LD100/7, i.e. up to that minimum dose, absolutely lethal for 100% of animals within 7 days. Using the integration method according to H. Behrens [6], in experiments on rats, the average lethal dose is calculated - $LD_{50/7}$.

At certain time ranges (days 3, 7, 21), blood was taken from the tail vein for haematological analysis.

The haematological parameters of experimental animals, namely the number of erythrocytes, leukocytes, platelets, hematocrit and haemoglobin content, as well as the blood clotting time, were determined using standard laboratory equipment [7].

Data analysis was carried out using a number of specialized statistical packages: StatSoft7, SPSS-10.0 and StatGraphicsPlus. We used regression and correlation methods of analysis.

RESULTS

The comparative analysis of survival and average life expectancy showed that complex **1** has the least toxicity of the two studied compounds. It should be noted that when complex **1** was administered subcutaneously to rats in the dose range from 480 mg/kg to 965 mg/kg, no disturbances in behavioural reactions and no animal death was observed. In the case of a higher dose (1450 mg/kg), immediately after the injection lethargy, retardation of movements, and decreased appetite were observed in some animals, which disappeared after 2-3 days. Death of only 1 animal (12,5%) was observed on the 4th day of monitoring, while in the remaining animals the mentioned phenomena disappeared and, until the end of the experiment, their behavioural reactions did not differ from those in intact animals. Based on the fact that previous toxicity experiments with low-toxic copper-containing chemical compounds at a dose of 1450 mg/kg showed the result of $LD_{50/7}$, and that the same dose of the bis[bis(3,5-dimethylpyrazol-1-yl)acetato]copper(II) complex (**1**) caused only death of the 12.5% of animals ($LD_{12.5/7}$), it can be concluded that the test substance is rather low-toxic, because a dose approximately 4 times higher was required to obtain $LD_{50/7}$. Thus, at a dose of 1450 mg/kg, we obtained a survival rate of 87,5%, and an average life expectancy of 26,8 days.

According to the test results, the $Cu_2(3,5\text{-DIPS})_4(H_2O)_3$ complex (complex **2**) turned out to be a low-toxic compound. It should be noted that with subcutaneous administration in the dose range from 100 mg/kg to 800 mg/kg, no disturbances in behavioural reactions and no animal death were observed within 24 hours. At a dose of 800 mg/kg, transient external signs of the suppressor effect of complex **2** on locomotor activity (physical inactivity) were noted in animals, which were no longer observed after 24 hours.

For complex **2** at a dose of 1450 mg/kg, survival was 75% and mean life expectancy was 22,9. This indicates that, even though both substances have very low toxicity, nevertheless, compared with complex **1**, the toxicity of complex **2** was higher and was characterized by lower values of those doses at which within 7 days after a single subcutaneous injection into the body 12,5% of animals will die (which is typical for complex **1**).

Figure 1 shows the dynamics of the survival of animals from two groups with complex **1** (surv1) and complex **2** (surv2) injections, with the corresponding regression curves and equations.

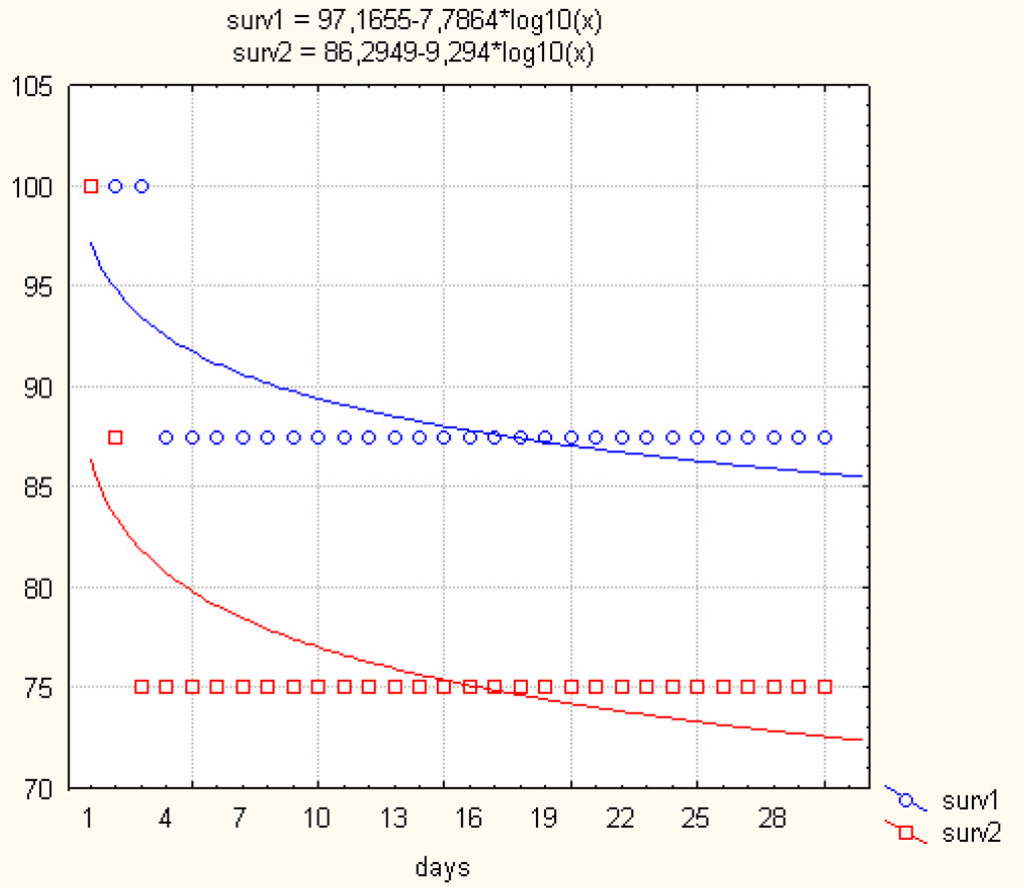

*Figure 1. Survival dynamics of animals from groups with complex **1** (surv1) and complex **2** (surv2) injections.*

Logarithmic regression equations were obtained: y_1 = 97,16-7,78lg(x) and y_2 = 86,29-9,29lg(x) (where y_1 is the survival of the group with complex **1** injection, y_2 is the survival of the group with complex **2** injection, and x is the number of days of the experiment), allowing not only to describe the dynamics of survival, but also, with the help of extrapolation, to predict a change in the percentage of survival in long term during the experiment.

An analysis of blood parameters was carried out on the 3rd and 7th days after subcutaneous administration of complex **1** at a dose of 1450 mg/kg. The results of haematological analysis are shown in Table 1. On the 3rd day of the study, an increase in the levels of all studied blood parameters was observed. This was followed by a decrease and, as can be seen in Table 1, on the 7th day there was a normalization of all blood indicators except for the number of platelets. Nevertheless, a trend towards normalization is also observed in this indicator.

A different picture was noted in the complex **2** injection group. According to the data in the Table, subcutaneous administration of complex **2** resulted in a significant decrease in haemoglobin levels, which was restored by the 7th day, and a significant decrease in blood clotting time, which persisted until the end of the study. By the 7th day, a significant decrease in the levels of platelets and erythrocytes was also noted.

Table 1: Results of hematological analysis

	Blood clotting time (sec)	**Leukocytes (N/μL)**	**Platelets (N/μL)**	**Erythrocytes (N/μL)**	**Haemoglobin (g/L)**	**Haematocrit (%)**
norm	311,0 ±19,00	11500,0 ±420,0	522000,0 ± 10560,0	5823000,0 ±278800,0	138,1 ±5,82	37,2 ±1,75

Day 3	(complex **1**)	320,0 ±16,22	14885,71 ±1248,13 (*)	654285,71 ±30945,97 (*)	6175714,29 ±361497,26	144,44 ±3,79	46,28 ±2,9 (*)
	(complex **2**)	200,8 ±9,95 (*)	10233,3 ±1178,04	577500,0 ±33609,27	4941666,7 ±444562,83	102,3 ±7,46 (*)	35,6 ±3,25
Day 7	(complex **1**)	283,0 ±12,44	13171,43 ±1790,15	634285,71 ±33263,53 (*)	5235714,29 ±270861,56	141,09 ±8,06	41,60 ±3,02
	(complex **2**)	249,0 ±12,36 (*)	10600,0 ±713,68	425833,3 ±20913,18 (*)	4575000,0 ±405421,37 (*)	139,5 ±3,84	45,4 ±4,18

(*) - at p<0.05 (when compared with control values, i.e. with a group of intact animals)

CONCLUSION

Thus, copper-based complexes $Cu[HC(COO)(pz^{Me2})_2]_2$ (complex **1**) and $Cu_2(3,5\text{-DIPS})_4(H_2O)_3$ (complex **2**) were shown to be low-toxic compounds. Only in the case of administration with ultra-high doses (more than 2000 mg/kg), immediately after the injection, lethargy and inhibition of animal movements were observed with subsequent death in a few hours.

Complex **1** is less toxic, because at a dose of 1450mg/kg, survival was 87,5% (versus 75% for complex **2**), mean duration of 26,8 (versus 22,9 for complex **2**).

Despite the multidirectional changes in blood parameters, which can be regarded as a manifestation of the development of intoxication, there was a tendency to normalize in both groups injected with complexes **1** and **2**. The difference is observed in the speed of recovery. The recovery of blood parameters in group with complex **1** injection is three times more rapid than in group treated with complex **2**.

The results of haematological studies also confirmed the lowest toxicity of complex **1**.

FUNDING

The work was supported by the Science Committee of RA, in the frame of the research project No 21T-1F126

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