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DETERMINATION OF THE TOXICITY OF A NEW COORDINATION COMPOUND WITH RADIATION PROTECTIVE ACTION

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Abstract: A new palladium and mexidol based complex coordination compound (NCC) – 2-Ethyl-6-Methyl-3-Hydroxypyridine ammonium Tetrachloropalladic Acid, synthesized at the Scientific Research Centre of Azerbaijan Medical University, has a radioprotective action. As such, it seems relevant to us, first of all, to study the toxicity of this compound, in particular, its acute and subchronic toxicity.

The aim of the research was to study the acute and subchronic toxicity of a new palladium and mexidol based complex coordination compound.

Materials and methods. The research was conducted on white, healthy, and sexually mature laboratory mice and rats. The acute toxicity was determined by using the Spearman-Karber method. Determination of subchronic toxicity was carried out on 10 male rats by using the LYM R.K. method.

Results. LD_{50} for new palladium and mexidol based complex coordination compound among the white laboratory mice is 355±184.471 mg/kg for male, and 385 mg/kg ±189.957 mg/kg for female mice, and it is 405 ±188.457mg/kg for male, and 430 ±187.227 mg/kg for female rats.

Keywords: acute toxicity, subchronic toxicity, palladium, mexidol, radioprotective property.

1. Introduction

Currently, the issue of the protection of human health from radiation is one of the most important and extremely urgent problems of practical pharmacology. Extended and more frequent contacts with the sources of ionizing radiation in the equipment of various kinds, application of ionizing radiation for cancer therapy purposes, the possibility of occurrence of emergency situations at nuclear power facilities and nuclear-powered plants, passenger aircraft flights at high altitudes, especially during solar flares, expected flights to other planets - all are the factors making it necessary to provide modern medicine with special drug preparations that reduce the effects of radiation exposure [1,2].

One of the most important elements of the medical radiation protection system for the preservation of human life and health is the preventive use of radioprotective substances. These are the preparations capable to increase for a short period the resistance of the human body against the effects of ionizing radiation [3]. It should be pointed out that despite the existence of positively proven and well-accepted radiation protection preparations of different chemical structures, the necessity of the search for new, more efficient, and less toxic compounds remains relevant. On this premise, prof. Hasanov H.I. obtained a new complex coordination compound of palladium (II) with mexidol based complex coordination compound at the premises of the Azerbaijan Medical University Research and Development Centre [4]. Palladium is a member of the platinum group of metals which exhibit antitumor activity. Mexidol, i.e. ethylmethylhydroxypyridine succinate, has antioxidant, anti-hypoxic, and membrane-protective properties. The preparation reduces the severity of lipid peroxidation, increasing at the same time the activity of the body's antioxidant defense system, and activating superoxide dismutase. The membrane-protective action of the drug is due to the reduction of the viscosity of the membrane

and an increase in its fluidity. It also increases the efficiency/activity of calcium-independent phosphodiesterase, adenylate cyclase, acetylcholinesterase, GABA receptors, acetylcholine, and other receptors [5, 6]. The study of new complex coordination compound of palladium (II) with mexidol and preliminary screening assays of biological potency have demonstrated its potential for medical applications, including its use in oncology practice. Taking into account the presence of mexidol, an antioxidant, and bivalent palladium, we assumed that this compound may have an antiradiation effect. As a result, the radiation protection action of NCC was confirmed based on the results of the experiments conducted on white non-pedigree rats, which were exposed to the radiation at the levels of 2.4 Gy and 6.2 Gy. The results obtained suggest that the preliminary administration of NCC against the background of irradiation with a power of 4 and 6.2 Gy, significantly reduces the harmful effect of on blood corpuscles [7]. This being the case, the study of the toxicity of this compound is of great importance in terms of medical and pharmaceutical sciences.

2. Materials and methods

The research was conducted on white, healthy, and sexually mature laboratory mice and rats obtained from a mouse breeding facility in Baku. They were quarantined in the vivarium of the Azerbaijan Medical University Research and Development Centre for 14 days. The acute toxicity (LD 50) of the substances for each species was determined separately for each sex. To prevent the distortion of the study indicators caused by daily and seasonal rhythms, a control group of the animals was formed, and the animals in that group were kept in identical conditions. The animals were divided into 7 groups. 1st group, i.e. the control group was divided into 2 subgroups, 5 animals in each subgroup. The 1st subgroup contained only male, and the 2nd group only had female animals. All animals in other groups were also divided into 2 subgroups (10 in each of the subgroups). The 1st subgroup contained only male, and the 2nd group only female animals, which were administered intraperitoneally the increasing doses of the test substance until the death of all animals. At the same time, tolerable, toxic, and lethal doses of the test substance were determined. The clinical presentation of intoxication was recorded, and the causes of death were determined.

The acute toxicity was determined by using the Spearman-Karber method. This nonparametric method elucidates the dose-response relationship and gives mathematical expectations and standard error values. In particular, this method allows us to quickly and reliably determine the average lethal LD50 doses (the dose at which a lethal outcome is observed in 50% of experimental animals) [8, 9].

Intervals between the administration of the doses across all groups were the same.

The doses of the substance were selected in such a way that 100% lethality of animals was observed in the group where the most concentrated solution was administered. That saying, this dose had to be at a 100%-lethality-ensuring level as a minimum. The group, where the animals were administered the most diluted solution of the test substance, should have zero lethality.

The pattern of dilution of the solution should be evenly distributed on a logarithmic scale.

The “dose-dilution” curve of the test substance should have a symmetric shape.

Only upon fulfillment of all these conditions will give reliable LD50 results with mean-square errors.

LD 50 = m=Xk - d (S_1-1/2), where

“Xk” is the minimum dose which leads to 100% lethality among the experiment animals;

“d” is the difference between the doses;

“S_1” is the total share of deaths.

The standard deviation is calculated using the following formula:

S LD 50 = Sm = $\frac{d\sqrt{252}}{}$ - S1-S2, where

"S_2" is the value indicating the sum of continuously summed up, accumulated share of deaths.

In this case, the confidence interval (maximum and minimum LD50 values) shall be calculated as follows: m ± 1.645 X Sm/

Determination of subchronic toxicity was carried out on 10 male rats using the Lym R.K. method. The animals received increasing doses of the test substance in accordance with the data calculated by this method. In essence, this method consists of the determination of the ratio of LD_{50} with a single injection to LD_{50} with multiple injections. It allows us to evaluate the cumulative properties of the test substance as well as the severity of the addiction it causes. The coefficient of cumulation or addiction is determined by the following formula:

$$K = \frac{\text{LD50 with a single injection}}{\text{LD50 with multiple injections}}$$

This being the case, if the value of the K coefficient is less than 1, the test substance has cumulative properties, while the same value exceeding 1 indicates an addiction.

The determination of cumulation allows for the calculation of the doses for administration in the process of a chronic experiment, and an adequate evaluation of obtained results.

3. Research results

The results of the determination of the acute toxicity through the study conducted on white non-pedigree laboratory mice are as follows:

In the 1st group, containing the intact animals (in both subgroups), no lethality cases were observed by the end of the 14-day observation period. All animals were active, with intact pelage without any damages, ulcers, etc. No pathological changes were recorded. The weights of the animals remained within 21±1.2 g among males, and 18±0.9 g among females. No pathomorphological changes in internal organs were recorded.

The results of the study are shown in table 1 below:

Table 1

Determination of LD50 doses in white laboratory male mice.

Group N=10	Test substance dose (mg/kg)	Number of dead animals	Share of dead animals	The cumulative share of dead animals
1st group	0	0	0	0
2nd group	250	0	0	0
3rd group	300	2	0.2	0.2
4th group	350	5	0.5	0.7
5th group	400	8	0.8	1.5
6th group	450	9	0.9	2.4
7th group	500	10	1.0	3.4
	Xк = 500		S1 = 3.4	S2 = 8.2

d- time interval between dose (50 mg/kg) administration

The animals receiving intraperitoneally increasing doses of the palladium and mexidol based test substance started to exhibit behavioral changes 15 minutes after the administration of

the dose. They had rapid breathing and increased motion activity. The animals' movements became slow after a while against the background of rapid breathing - they huddled in the corner of the cage and stopped moving. With increased doses of the test substance, the mortality among the animals increased starting from the 3rd group.

Based on the obtained results, we calculated the value of LD50 among the white laboratory male mice, which have received palladium and mexidol based complex coordination compound solutions of different dilution levels using the Spearman-Karber method:

LD50 = 500 -50 (3.4-0.5) = 355 mg/kg;

Thus, the LD50 dose of new palladium and mexidol based complex coordination compound for white laboratory male mice is 355 mg/kg.

Let's calculate the standard deviation for the obtained LD50 value:

Sm =50 √ 2 x8.2 -3.4 -3.42 = 50 x4.01 -3.4-11.56 -0.083 = 184. 471;

The 90% confidence interval of the LD50 dose of new palladium and mexidol based complex coordination compound for white laboratory male mice shall have the following values: m ± Sm

Thus,

m max = 355 + 184.471 = 539.471 mg/kg

m min = 355 – 184.471 = 170.529 mg/kg

The results of the determination of the acute toxicity through the study conducted on white non-pedigree laboratory female mice are shown in table 2.

Table 2

Determination of LD50 in female white laboratory mice

Group N=10	Test substance dose (mg/kg)	Number of dead animals	Share of dead animals	The cumulative share of dead animals
1st group	0	0	0	0
2nd group	280	0	0	0
3rd group	330	3	0.3	0.3
4th group	380	5	0.5	0.8
5th group	430	7	0.7	1.5
6th group	480	9	0.9	2.4
7th group	530	10	1.0	3.4
	Xк = 530		S1 =3.4	S2 =8.4

d- time interval between dose (50 mg/kg) administration.

Calculation of the value of LD50 among the white laboratory male mice, which have received palladium and mexidol based complex coordination compound solutions of different dilution levels using the Spearman-Karber method:

LD50= 530 -50 (3.4-0.5) = 385 mg/kg;

Thus, the LD50 dose of new palladium and mexidol based complex coordination compound for white laboratory female mice is 385 mg/kg.

The standard deviation for the obtained LD50 value:

Sm =50 √ 2 x8.4 -3.4 -3.42 = 50 x4.1 -3.4-11.56 -0.083 = 189.957;

The 90% confidence interval of the LD50 dose of new palladium and mexidol based complex coordination compound for white laboratory female mice is: m ± Sm

Thus,
m max = 355 + 189.957 = 544.957 mg/kg
m min = 355 – 189.957 = 165.043 mg/kg

In the 1st group of the rats (in both subgroups) all animals were active, with the water and food consumption remaining within normal range; and with intact pelage without any damages, ulcers, etc. were recorded. The weights of the animals remained within 230±5.6 g among males, and 190±7.3 g among females. No pathomorphological changes in internal organs were recorded.

The results of the determination of the acute toxicity through the study conducted on white non-pedigree laboratory rats are shown in table 3.

Table 3

Determination of LD50 in male white laboratory rats

Group N=10	Test substance dose (mg/kg)	Number of dead animals	Share of dead animals	The cumulative share of dead animals
1st group	0	0	0	0
2nd group	300	0	0	0
3rd group	350	2	0.2	0.2
4th group	400	6	0.6	0.8
5th group	450	7	0.7	1.5
6th group	500	9	0.9	2.4
7th group	550	10	1.0	3.4
	Xк = 550		S1 = 3.4	S2 = 8.3

d- time interval between dose (50 mg/kg) administration.

Calculation of the value of LD50 among the white laboratory male rats, which have received palladium and mexidol based complex coordination compound solutions of different dilution levels using the Spearman-Karber method:

LD50 = 550 -50 (3.4-0.5) = 405 mg/kg;

Thus, the LD50 dose of new palladium and mexidol based complex coordination compound for white laboratory male rats is 405 mg/kg.

The standard deviation for the obtained LD50 value:

Sm =50 √ 2 x8.3-3.4 -3.42 = 50 x4.07 -3.4-11.56 - 0.083 = 188.457;

The 90% confidence interval of the LD50 dose of new palladium and mexidol based complex coordination compound for white laboratory male rats shall have the following values: m ± Sm

Thus,
m max = 405 + 188.457 = 593.457 mg/kg
m min = 405 – 188.457 = 216.543 mg/kg

The results of the determination of the acute toxicity through the study conducted on white non-pedigree laboratory female rats are shown in table 4.

Table 4

Determination of LD50 in female white laboratory rats.

Group N=10	Test substance dose (mg/kg)	Number of dead animals	Share of dead animals	The cumulative share of dead animals
1st group	0	0	0	0
2nd group	320	0	0	0
3rd group	370	2	0.2	0.2
4th group	420	6	0.6	0.8
5th group	470	7	0.7	1.5
6th group	520	8	0.8	2.3
7th group	570	10	1.0	3.3
	Xk = 570		S1 = 3.3	S2 = 8.1

d- time interval between dose (50 mg/kg) administration.

The study, whose results are presented in Table 4, demonstrates that in the second group, 20 minutes after the administration of 320 mg/kg of the test substance intraperitoneally, signs of intoxication are observed, which disappear after a day, according to visual observations. And later on, the animals achieve full rehabilitation, with their observed indicators eventually reaching the values of the intact group. No lethal cases occurred in the 2nd subgroup. The weights of the animals remained within 230±6.4 g among males.

With increased doses of the administered test substance, intoxication signs, and mortality among the following groups increased in the following groups.

Calculation of the value of LD50 among the white laboratory female rats, which have received palladium and mexidol based complex coordination compound solutions of different dilution levels using the Spearman-Karber method:

LD50 = 570 -50 (3.3-0.5) = 430 mg/kg;

Thus, the LD50 dose of new palladium and mexidol based complex coordination compound for white laboratory female rats is 430 mg/kg.

The standard deviation for the obtained LD50 value:

Sm =50 √ 2 x8.1-3.3-3.32 = 50 x4.03 -3.3-10.89 -0.083 = 187.227;

The 90% confidence interval of the LD50 dose of new palladium and mexidol based complex coordination compound for white laboratory female rats is: m ± Sm

Thus,

m max = 430 + 187.227 = 617.227 mg/kg

m min = 430 – 187.227 = 242.773 mg/kg

Macroscopic examination of the internal organs of dead animals.

Corpses of dead animals from all groups were dissected in the process of the acute toxicity study, and their internal organs were subjected to visual examination and macroscopic evaluation.

The analysis of the post-mortem dissection of all female and male rats and mice revealed that there aren't any pathological fluids or commissures in the thoracic cavity, while there was a certain amount of accumulated reddish-yellow fluid.

Oral cavity examination: the tongue has a dark coating, the mucous membrane of the larynx has a natural pink color, and is moist. Almost no differences were found from intact animals according to these indicators.

Respiratory organs: trachea and bronchi are without pathologies, and mucous membranes are moist, shiny, and smooth. The lungs are airy and have a grey-pink color. The pleura is thin, within the normal range. Some species have induration areas in their lower parts.

Heart: No deviations from the norm were revealed. It has a standard rounded shape, and heart contraction during the systole period is good. The pattern of coronary vessels under the epicardium is clearly visible and physiologically normal. The aorta is elastic, the intima is clean and smooth.

Abdominal cavity organs: the esophagus and stomach are hyperemic and edematous.

The liver is enlarged, flabby, yellow-brown in section, with hemorrhages under the capsule. The spleen is gray and the splenic capsule is smooth, with multiple hemorrhages under it. The intestines are hyperemic and edematous. The mucous membrane forms lateral folds. It is moist but has a gray-black color. The kidneys' locations are symmetric, and a dark surface is revealed after the removal of the capsule with multiple hemorrhages. The kidneys have clearly distinguished layers in the section. The mucous membrane of the pelvis, and bladder is dark gray, dull, and dry. The adrenal bodies are enlarged, and rounded, with a clearly visible division of the parenchyma into the cortical and medulla zones.

As can be seen from the above, the macroscopic examination of the thoracic and abdominal cavities of the dead animals from all groups revealed nonsurvivable changes. The liver and kidneys were especially affected. The body masses of the experimental animals by the end of the observation period were directly proportional to the increased doses of administered test substance. The pelage of surviving mice and rats in the experimental groups was undamaged.

Determination of subchronic toxicity allows for revealing the cumulative properties of the substance. The results of the study are shown in table 5 below:

Table 5

Determination of the accumulation of a complex compound based on palladium and mexidol by the method of subchronic toxicity according to Lym.

№	Days of substance administration	Share of LD50	Cumulative dose(LD50;n) mg/kg	Number of dead rats	Number of surviving rats
1	**1**	**0.1 =40 mg/kg**	**40**	**0**	**10**
	2	**0.1**	**80**	**0**	**10**
	3	**0.1**	**120**	**0**	**10**
	4	**0.1**	**160**	**0**	**10**
2	**5**	**0.15= 60 mg/kg**	**220**	**0**	**10**
	6	**0.15**	**280**	**0**	**10**
	7	**0.15**	**340**	**0**	**10**
	8	**0.15**	**400**	**0**	**10**
3	**9**	**0.22= 90 mg/kg**	**490**	**1**	**9**
	10	**0.22**	**580**	**1**	**9**
	11	**0.22**	**670**	**5**	**5**
	12	**0.22**	**760**	**3**	**2**
4	**13**	**0.34= 135mg/kg**	**895**	**2**	**0**

To calculate the cumulation coefficient $K_к$:

$K_к = LD50/LD50_n = 400/ 895 = 0.44$;

$K_к$ <1 shall mean that the test substance accumulates in the body.

4. Conclusions

LD_{50} for new palladium and mexidol based complex coordination compound among the white laboratory mice is 355±184.471 mg/kg for male, and 385 mg/kg ±189.957 mg/kg for female mice, and it is 405 ±188.457mg/kg for male, and 430 ±187.227 mg/kg for female rats.

According to Hodge and Sterner toxicity scale (1943), NCC belongs to a moderately toxic group, while according to the classification of chemical substances under GOST 12.1.007-76, it refers to Hazard Class 3, which is authorized for medical use.

The macroscopic examination of the thoracic and abdominal cavity organs revealed nonsurvivable changes. The liver and kidneys were especially affected.

It was established that the body masses of the experimental animals by the end of the observation period were directly proportional to the increased doses of administered test substance. The pelage of surviving mice and rats in the experimental groups was undamaged.

Determination of the cumulation by the Lym method revealed that the cumulation coefficient is $K_к$ <1. This means that the test substance accumulates in the animal body.

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ОПРЕДЕЛЕНИЕ ТОКСИЧНОСТИ НОВОГО КОМПЛЕКСНОГО СОЕДИНЕНИЯ С РАДИОЗАЩИТНЫМ ДЕЙСТВИЕМ

Н.Ф. Магеррамова

Резюме: Синтезированный на базе НИЦ АМУ новый комплекс на основе палладия с мексидолом (НКС) -2-этил-6-метил-3-гидроксипиридин аммоний тетрахлоропалладиевокислый, обладает радиозащитным действием. В этой связи нам представляется актуальным в первую очередь изучение токсичности данного соединения, в частности острую и субхроническую токсичность.

Целью исследования является изучение острой и субхронической токсичности нового комплексного соединения на основе палладия и мексидола.

Материалы и методы. Исследования проводили на здоровых половозрелых белых лабораторных мышах и крысах. Острую токсичность определяли методом Спирмана–Кербера. Определение субхронической токсичности проводили на 10 крысах-самцах по методу Lim R.K.

Результаты. LD_{50} для нового комплексного соединения на основе палладия и мексидола для белых лабораторных мышей - самцов составляет 355±184,471 мг/кг, а для самок 385 мг/кг ±189,957 мг/кг; для белых лабораторных крыс-самцов 405±188,457 мг/кг, крыс -самок составляет 430 ±187,227 мг/кг.

Ключевые слова: острая токсичность, субхроническая токсичность, палладий, мексидол, радиозащитное свойство.

RADİOPROTEKTİV TƏSİRƏ MALİK YENİ KOMPLEKS BİRLƏŞMƏNİN TOKSİKLİYİNİN TƏYİNİ

N.F. Məhərrəmova

Xülasə: ATU-nun Elmi-Tədqiqat Mərkəzində sintez edilmiş palladium və meksidol əsaslı (-2-etil-6-metil-3-hidroksipiridin ammonium tetraxloropalladium turşusu) yeni kompleks radioprotektiv təsirə malikdir. Bu baxımdan, ilk növbədə, bu birləşmənin toksiklik dərəcəsini, xüsusən də kəskin və subxronik toksikliyini öyrənməyi aktual hesab edirik.

Tədqiqatın məqsədi palladium və meksidol əsasında yeni kompleks birləşmənin kəskin və subxronik toksikliyini öyrənməkdir.

Materiallar və metodlar. Tədqiqatlar sağlam yetkin ağ laboratoriya siçanları və siçovulları üzərində aparılıb. Kəskin toksiklik Spirman - Kerber metodu ilə müəyyən edilmişdir. Subxronik toksikliyin təyini R.K. Lym metodu ilə 10 erkək siçovul üzərində aparılmışdır.

Nəticələr. Erkək ağ laboratoriya siçanları üçün palladium və meksidol əsaslı yeni kompleks birləşmə üçün LD_{50} - 355±184,471 mq/kq, dişilər üçün isə 385 mq/kq ±189,957 mq/kq; ağ laboratoriya erkək siçovulları üçün 405 ±188,457 mq/kq , dişi siçovullar üçün isə 430 ±187,227 mq/kq təşkil edir.

Açar sözlər: kəskin toksiklik, subxronik toksiklik, palladium, meksidol, radioprotektiv xüsusiyyət.