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THE STUDY OF THE SAFETY PROFILE OF “ALTSINARA” DRUG UNDER THE CONDITIONS OF ACUTE TOXICITY MODELING

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The results of the preclinical safety study of “Altsinara” drug, tablets for oral use produced by JSC SPC “Borschagovsky CPP” private joint stock company are given. While studying acute toxicity it has been determined that in a single intragastric administration it does not have toxic effects on the general condition and behaviour of animals, do not cause their death, as well as visible changes in the internal organs. The absence of lethality when introducing “Altsinara” suggests that the LD_{50} value for this drug exceeds the maximum dose used in the experiment, i.e. LD_{50} of “Altsinara” is more than 5000 mg/kg by the amount of active substances in intragastric introduction. According to the conventional classification of K.K.Sidorov this LD_{50} value of “Altsinara” drug can be referred to the V class of toxicity – practically non-toxic substances in the route of administration studied. Thus, the results of the study indicate that “Altsinara” is the drug, which is practically safe for a human, almost has no toxic effects on the body with a single administration.

Over 4 billion people or 80% of the world's population use herbal medicines as their source of primary care [15]. Thus, today, almost 25% of all drugs, which are available at the pharmaceutical market and widely used in clinical practice in different pharmaceutical formulations, contain ingredients from plants [14]. Hence, one of the problems of the modern science and practice of pharmacy and medicine is to provide further integration of safe herbal medicines into the treatment process.

Pharmacological and toxicological evaluations of herbal drugs are essential for drug development. Even though much is done in screening herbal medicines for efficacy based on the traditional approach, the problem of safety and toxicity is less emphasized.

In order to meet the needs of our population for quality medicines a new domestic combination of the herbal origin in a tablet dosage form “Altsinara” has been developed by JSC SPC “Borschagovsky CPP”.

“Altsinara” contains the extract of artichoke leaves and the garlic powder. This composition suggests the presence such pharmacological actions as hepatoprotective,

choloretic, hypolipidemic, nephroprotective, diuretic and other elements of the pharmacodynamics spectrum in this drug at a high level of safety for patients. The use of a drug with a similar complex of pharmacological properties can be promising in prevention and treatment of variety of diseases of the cardiovascular, hepato-biliary and urinary systems [1, 3, 5, 11, 13, 14].

The aim of this study was to conduct an experimental study of the safety profile (acute toxicity) of “Altsinara” drug in tablets for oral use produced by JSC SPC “Borschagovsky CPP”.

Materials and Methods

Preclinical studies of the parameters of acute toxicity for “Altsinara” were conducted by the method of V.B. Prozorovskiy [6]. It was introduced intragastrically to 36 non-linear white rats of both sexes weighing 150-180 g. The rats were divided into 6 experimental groups of 6 animals in each group: group 1 – animals treated with “Altsinara” drug in the dose of 500 mg/kg; group 2 – animals received “Altsinara” in the dose of 1.000 mg/kg; group 3 – animals treated with “Altsinara” in the dose of 2.000 mg/kg; group 4 – animals

received “Altsinara” in the dose of 3.000 mg/kg; group 5 – animals treated with “Altsinara” in the dose of 4.000 mg/kg; group 6 – animals treated with “Altsinara” in the dose of 5.000 mg/kg. In all cases the doses were by the amount of active substances. If the total amount of the suspension contained more than 5 ml of the drug studied per one animal, then “Altsinara” was introduced fractionally during the day [2, 8].

Considering that the mass production of “Altsinara” was intended as coated tablets the intragastric route of administration by gavage was chosen. The toxicity class of “Altsinara” was determined according to the conventional classification of K.K.Sidorov [10].

During the study (14 days) there were observations on survival of the experimental animals, food and water consumption, as well as the clinical signs of intoxication (if they occurred) by the general condition, changes in posture, skin, the colour of the mucous membranes, the body temperature and individual symptoms (miosis, watery diarrhea, discoloration of urine and faeces, drowsiness, seizures, etc.).

In case of death the animals were subjected to autopsy and macroscopic analysis of the abdomen in order to determine that the death was due to manipulation errors,

Table 1

The mortality parameters of “Altsinara” when studying acute toxicity in intragastric introduction (n=36)

Group	Dose of “Altsinara”, mg/kg	Number of rats	The number of dead rats				
			Day 1	Day 4	Day 7	Day 10	Day 14
1	500	6	0	0	0	0	0
2	1000	6	0	0	0	0	0
3	2000	6	0	0	0	0	0
4	3000	6	0	0	0	0	0
5	4000	6	0	0	0	0	0
6	5000	6	0	0	0	0	0

as well as to identify the probable cause of death.

In addition, the internal organs were weighed (brain, heart, kidneys, liver, spleen), and their mass ratios were determined.

To calculate the average lethal dose (LD_{50}) the percentage of mortality in each group was determined in 14 days and with the help of tables, and LD_{50} values were determined by calculations using the method of probit analysis of mortality curves according to V.B. Prozorovskiy [6].

The statistical processing of the results obtained was carried out by methods of variation statistics by Student t-test and non-parametric methods of analysis (Mann-Whitney U Test) using such computer programmes as STATISTICA 7.0, StatPlus 2009 and MS Excel 2007 [4, 7, 9] and represented as a comparative tables with the results of different groups.

Results and Discussion

According to the results of studying the acute toxicity of “Alzinar” drug in intragastric introduction to rats in the doses of 500 and 1.000 mg/kg no visible signs of toxic effects of this drug on the functional condition of the animals were registered. Rats quietly endured the introduction of the drug under research, and their appearance, behaviour and general condition remained unchanged. Further observations during the day did not reveal abnormalities in their general condition and behaviour.

After introduction of “Altsinara” in the doses of 2.000 and 3.000 mg/kg to rats some weakness, lethargy, decreased physical activity, decreased appetite were noted in the first hour of observation. Over the next 2-3 hours the general condition and behaviour of animals approached to the physiological normal ranges, and all manifestations of intoxication disappeared.

In contrast, after introduction of “Altsinara” in the doses of 4.000 and 5.000 mg/kg moderate signs of intoxication were observed. Due to the fact that the total number of the drug per an animal reached 10.0 ml, which was twice allowable amount of administration, the drug was introduced in three parts with an interval of two hours. Significant signs of toxicity were observed in most cases only after the third introduction and continued to become more pronounced. All rats were in the sedentary condition, the response to external irritants was reduced, and breathing was accelerated. This state was observed until the end of the day, and the next day the animals began to move in the cage drinking water and eating. In addition, manifestations of pronounced diarrhea were observed in rats, it might be associated not only with the action of the drug, but the excessive amounts of liquid introduced as part of the suspension of the drug studied. The overall functional status of rats completely approached the physiological norm by the end of the second day of observation.

During the study there were no cases of mortality of animals in the experimental groups (Table 1).

During the research the body weight of rats was also monitored. It was determined that the body weight increased by 8.0% on average, but it was not of a reliable nature compared with the original data. Thus, no negative impact on the body weight gain of animals was observed. On day 14 of the experiment after euthanasia of rats the autopsy studies of the abdomen and brain were conducted. During the macroscopic study of the internal organs all animals were examined for the presence of visible pathological signs. According to the results of analysis significant deviations from the norm were not observed. Visible mucous membranes were shiny, pink, straight, without deviation from the normal ranges, the lymph nodes were not enlarged, with the normal location of the organs, adhesions were observed. All macroscopically screened organs (brain, heart, kidneys, liver, spleen) were of normal size, colour and density of tissues. When calculating and analysing the indicators of mass ratios of the internal organs of animals the probable dynamic changes were not observed – all parameters were within the physiological normal ranges (Table 2).

The absence of mortality in laboratory animals in intragastric introduction of “Altsinara” suggests that the LD_{50} value for the given drug exceeds the maximum dose

Table 2

Mass ratios of the internal organs of rats when studying acute toxicity of "Altsinara" in intragastric introduction (n = 36)

Group	Dose of "Altsinara", mg/kg	Mass ratio, %				
		Brain	Heart	Kidney (right)	Spleen	Liver
1	500	1.00±0.03	0.40±0.01	0.32±0.01	0.38±0.01	3.45±0.09
2	1000	1.04±0.03	0.44±0.01	0.30±0.01	0.41±0.01	3.44±0.09
3	2000	0.99±0.03	0.41±0.01	0.32±0.01	0.39±0.01	3.48±0.08
4	3000	1.03±0.03	0.44±0.01	0.33±0.01	0.40±0.01	3.42±0.09
5	4000	1.07±0.03	0.42±0.01	0.32±0.01	0.39±0.01	3.43±0.09
6	5000	1.04±0.03	0.46±0.01	0.33±0.01	0.42±0.01	3.47±0.09

used in the experiment, i.e. LD₅₀ of "Altsinara" > 5000 mg/kg in intragastric introduction in rats (by the amount of active substances). This LD₅₀ value allows to refer "Altsinara" drug with the route of administration studied to the V class of toxicity – practically non-toxic substances according to the conventional classification of K.K.Sidorov.

Thus, the results of the study indicate that "Altsinara" drug in a single intragastric administration does not practically have toxic effects on the general condition, be-

haviour, food intake and the body weight of animals; has no effect on the absolute and relative mass of internal organs of animals; does not cause visible changes in the internal organs of animals; does not cause the death of animals; refers to the fifth class of toxicity – practically non-toxic substances.

CONCLUSIONS

1. In a single intragastric introduction "Altsinara" drug in rats in the dose range of 500-5000 mg/kg by the amount of active substances does not have any toxic effects

on the general condition and behaviour of animals and does not cause their death. This fact suggests that in the given route of administration the LD₅₀ value of the drug exceeds 5000 mg/kg.

2. According to the toxicological characteristics "Altsinara" drug is considered to be practically non-toxic to the human body since the LD₅₀ values obtained according to the conventional classification of K.K.Sidorov can be referred to the V class of toxicity – practically non-toxic substances in the route of administration studied.

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ВИВЧЕННЯ БЕЗПЕКИ ПРЕПАРАТУ «АЛЬЦИНАРА» ЗА УМОВ МОДЕЛЮВАННЯ ГОСТРОЇ ТОКСИЧНОСТІ

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Ключові слова: препарат; «Альцинара»; токсикологічні властивості; гостра токсичність

Наведені результати доклінічного дослідження безпеки препарату «Альцинара», таблетки для перорального застосування виробництва ПАТ НВЦ «Борщагівський ХФЗ». У ході досліджень гострої токсичності даного засобу було визначено, що за умов внутрішньошлункового одноразового введення він практично не чинить токсичного впливу на загальний стан та поведінку тварин, не викликає їх загибелі, а також видимих змін у внутрішніх органах. Відсутність летальності при введенні препарату «Альцинара» дозволяє вважати, що значення LD_{50} для даного препарату перевищує максимальну дозу, яку використовували в експерименті, тобто при внутрішньошлунковому введенні LD_{50} Альцинари > 5000 мг/кг за сумою діючих речовин. Таке значення LD_{50} дозволяє віднести препарат при дослідженому шляху введення за загальноприйнятою класифікацією К.К. Сидорова до V класу токсичності – практично нетоксичні речовини. Таким чином, результати проведених досліджень свідчать про те, що «Альцинара» – препарат практично безпечний для людини, що майже не чинить токсичного впливу на організм при одноразовому застосуванні.

ИЗУЧЕНИЕ БЕЗОПАСНОСТИ ПРЕПАРАТА «АЛЬЦИНАРА» В УСЛОВИЯХ МОДЕЛИРОВАНИЯ ОСТРОЙ ТОКСИЧНОСТИ

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Приведены результаты доклинического исследования безопасности препарата «Альцинара», таблетки для перорального применения производства ПАО НПЦ «Борщаговский ХФЗ». В ходе исследований острой токсичности данного средства было определено, что при внутрижелудочном однократном введении оно практически не оказывает токсического воздействия на общее состояние и поведение животных, не вызывает их гибели, а также видимых изменений во внутренних органах. Отсутствие летальности при введении препарата «Альцинара» позволяет считать, что значение LD_{50} для данного препарата превышает максимальную дозу, которую использовали в эксперименте, то есть при внутрижелудочном введении LD_{50} Альцинары > 5000 мг/кг по сумме действующих веществ. Данное значение LD_{50} позволяет отнести препарат при исследованном пути введения по общепринятой классификации К.К. Сидорова к V классу токсичности – практически нетоксичные вещества. Таким образом, результаты проведенных исследований свидетельствуют о том, что «Альцинара» является препаратом практически безопасным для человека, почти не оказывает токсического воздействия на организм при однократном применении.

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