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THE RESULTS OF THE ACUTE TOXICITY STUDIES OF "DOLOSAN FORTE®" TABLETS

This study aimed to evaluate the parameters of acute toxicity of the new combined tablets "Dolosan Forte®" (containing zirilon – 2,4-dichlorobenzoic acid potassium salt, pitofenone hydrochloride, fenpiverinium bromide) worked out by PJSC "Red Star". It has been shown that LD_{50} for these tablets administered intragastrically equals 13231 ± 2775 mg/kg in male rats, 13216 ± 3379 mg/kg in female rats, 14393 ± 1669 mg/kg in male mice and 11963 ± 2023 mg/kg in female mice, corresponding to the V class of practically nontoxic substances (5000 mg/kg < LD_{50} < 15000 mg/kg). The death of the rats and mice receiving the investigated tablets can be possibly caused by the neurotoxic action of the drug with the rapid development of convulsions. The survived animals demonstrated normal state during 14 days. Macroscopic examination showed the absence of the morphological signs of the toxic effect of "Dolosan Forte®" tablets on the visceral systems of the survived rats and mice (except for the increment of the relative organ weights in the groups of animals receiving doses causing high lethality rate). Microscopic investigation did not reveal any pathological changes of the internal organs of rats after the single intragastric administration of "Dolosan Forte®" tablets at a dose of 6000 mg/kg. There were no interspecies as well as sex-specific differences in "Dolosan Forte®" toxic effects. The obtained results together with the data in the literature concerning LD_{50} for the components of "Dolosan Forte®" show that combined use of zirilon (2,4-dichlorobenzoic acid potassium salt), pitofenone hydrochloride, fenpiverinium bromide does not cause the increase in their acute toxicity.

Key words: acute toxicity; zirilon (2,4-dichlorobenzoic acid potassium salt); pitofenone hydrochloride; fenpiverinium bromide; combination; rats; mice

INTRODUCTION

Pain remains the common healthcare problem being one of the leading reasons for consulting a doctor. It significantly impairs quality of life and ability to work, still the effective treatment of chronic pain (which exists in the absence of tissue damage) remains suboptimal and often causes side effects and excessive healthcare costs [9, 13]. So, there is a need to search for the new drugs with the high level of safety as the widely known analgesics such as sodium metamizole are able to cause blood dyscrasias and other side effects, for this reason their use is limited [10]. Zirilon (2,4-dichlorobenzoic acid potassium salt) is the promising substance in this context as it is an effective antiinflammatory agent and analgesic with the therapeutic indices significantly exceeding the values of diclofenac sodium and metamizole sodium [6].

Spasm is among the leading reasons of pain, it also contributes to the pathogenesis of the different diseases worsening the blood supply to the organs and tissues. One of the rational approaches to the pharmacological correction of pain and spasm is to combine analgesics with spasmolytics. It is expedient to use zirilon together with the spasmolytics possessing the different mechanisms of action such as pitofenone hydrochloride (myotropic spasmolytic) and fenpiverinium bromide (M-cholinolytic with additional myotropic and analgesic properties). Such combination – the tablets "Dolosan Forte®" – was worked out by PJSC "Red Star" (Kharkiv, Ukraine). In the previous experiments [5] it has been shown that this combined drug is effective in doses lower than the reference drug "Spasmalgon®" and has the advantage over the latter being indifferent to the normal intestinal motility.

Besides, there is evidence in the literature that pitofenone counteraction to spasm is clearly manifested *in vitro* in simultaneous use with antiinflammatory agents [11]. It was shown in clinical studies that diclofenac combined with pitofenone and fenpiverinium effectively reduces the pain intensity in biliary, ureteric, and intestinal colic demonstrating synergistic action [8].

Therefore, further studies of the combination of zirilon with pitofenone hydrochloride and fenpiverinium bromide is expedient. Toxicity determination represent an essential part of the preclinical drug research and is absolutely necessary for the new combined drugs. Therefore, the aim of this study was to establish the parameters of acute toxicity of the tablets "Dolosan Forte®".

MATERIALS AND METHODS

Adult random-bred mice with body weight of 22-26 g (females, n = 36) and 16-19 g (males, n = 36) as well as adult random-bred rats of both sexes (n = 36 in each group) with body weight of 170-200 g were kept in the Central Scientific-Research Laboratory of National University of Pharmacy under standard conditions. All the experimental protocols corresponded to the requirements of the "Directive 2010 / 63 / EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes."

As the peroral route of administration is assumed for the tablets "Dolosan Forte®" and is able to provide the systemic action, it was used in the experiments to reproduce the clinical signs of the acute poisoning and to determine LD_{50} value. The investigated drug was administered once in the form of water suspension (the amount equalled 0.2 ml/10 g for mice and 2 ml/100 g for rats), the animals of the intact control groups received water by the similar scheme. Before the administration the animals were fasted for 12 h (rats) and 4 h (mice), after the administration – for 4 h, but they were allowed free access to water. The animals were observed for 14 days after dosing, body weight dynamics, food and water consumption were also registered. After this the animals were anesthetized and the internal organs were harvested for morphological studies and determination of relative organ weight [2]. In accordance with [1, 3] internal organs (namely the liver, kidneys, heart, adrenal glands, spleen, thymus, testicles / ovaries, as well as oesophagus, stomach, jejunum and rectum, that directly contacted with the drug) were harvested for morphological studies. The organs were fixed in 10 % solution of neutral formalin, dehydrated in ethanol solutions of the increasing concentration, embedded in celloidin-paraffin. Microtome sections were stained with hematoxylin and eosin [5]. For the analysis the microscope Granum L3000 with the appropriate camera and software were used.

Calculation of LD_{50} was performed by the method of V. B. Prozorovskiy [2]. The other data were processed using the standard software "Statistica 6.0", dispersion analysis, Newman-Keuls criterion, Kruskal-Wallis method, Mann-Whitney criterion were applied. The level of significance was defined as $p \leq 0.05$.

RESULTS AND DISCUSSION

1. The results obtained in experiments in rats

It was established that 10 min after the single intragastric administration of the tablets "Dolosan Forte®" there was a decrease in the locomotor activity, then lateral position was registered, and convulsions developed in the most of the rats. Against the background of the drug at doses 10000 mg/kg and higher, extremely severe convulsions were seen, epistaxis occurred in some cases, and death was observed within 20-30 min. Such clinical symptoms are the evidence of CNS affection. The condition of the survived animals improved gradually after 30 min, still adynamia persisted. All these signs disappeared the next day, the condition of the animals did not have any differences from intact rats.

Table 1

THE RESULTS OF THE ACUTE TOXICITY STUDIES OF THE TABLETS "DOLOSAN FORTE®" IN RATS USING INTRAGASTRIC ADMINISTRATION

Groups		Dose, mg/kg	The effect observed, dead animals/number of animals in the group	
			males	females
1	Intact control	–	0/6	0/6
2	The tablets "Dolosan Forte®"	6000	0/6	0/6
3		10000	1/6	2/6
4		14000	3/6	3/6
5		16000	5/6	4/6
6		18000	6/6	6/6

The data listed in Tab. 1 allowed calculating of LD_{50} value for the tablets "Dolosan Forte®" administered intragastrically to rats: it equals 13231 ± 2775 mg/kg in males and 13216 ± 3379 mg/kg in females.

Subsequently, the observation on the animals survived during 14 days shown that they demonstrated normal activity, appearance, and appetite, responded to auditory and visual stimuli; processes of urination and defecation were normal; respiratory disorders, and seizures were not observed. Macroscopic examination on day 14 did not show any pathological changes in animal's general state of health and well-being. The consumption of food in all the experimental animals did not differ from those in the groups of intact control, water consumption had some inter-individual differences without any consistent patterns between the groups. There was a positive dynamics in the body weight dynamics in all of the groups without any differences.

Still in some of the experimental groups higher increment in the body weight was seen, namely among the male rats receiving the investigated drug at doses of 14000 and 16000 mg/kg and among the female rats receiving it at a dose of 10000 mg/kg. This may be determined by the low number of animals in the mentioned groups due to lethality.

Macroscopic examination of the animals survived during 14 days showed no differences from intact rats in the general appearance, as well as in macroscopic structure of the thoracic and abdominal cavities, lymph nodes, thymus, heart and its valves, lungs, stomach (including mucous membrane structure), intestine and colon, liver, pancreatic gland, spleen, adrenal glands, kidneys and bladder uterus and gonads. There were no changes in the relative organs weights (namely, the liver, kidneys, heart, lungs, spleen, thymus, adrenal glands, testes) in male rats receiving the tablets "Dolosan Forte®" at doses of 6000 and 10000 mg/kg, the increase in the spleen relative weight was seen in males receiving the drug at a dose of 14000 mg/kg.

Table 2

THE RESULTS OF THE ACUTE TOXICITY STUDIES OF THE TABLETS "DOLOSAN FORTE®" IN MICE USING INTRAGASTRIC ADMINISTRATION

Groups		Dose, mg/kg		The effect observed, dead animals/ number of animals in the group	
		males	females	males	females
1	Intact control	–	–	0/6	0/6
2	The tablets "Dolosan Forte®"	10000	10000	0/6	0/6
3		12000	11500	1/6	3/6
4		14000	12500	3/6	4/6
5		16000	15000	4/6	5/6
6		18000	17500	6/6	6/6

In all of the groups of female rats, the relative organs weights (mentioned above, except for testes) were within the normal physiological range.

Microscopic investigation was performed for the samples of the rats receiving the tablets "Dolosan Forte®" at a dose of 6000 mg/kg. Compared with the intact animals, no pathological changes were revealed in the histological structure of the myocardium, lungs and bronchial epithelium, liver, kidneys, adrenal glands, spleen, thymus, pancreas, testicles and ovaries (spermatogenesis and oogenesis processes were unchanged). There were no pathological shifts in the place of the primary contact with the drug – the gastrointestinal system (namely, the oesophagus mucous membrane, gastric mucosa in all parts, intestinal epithelium of the jejunum and pelvic colon).

Therefore, the results of the macroscopic examination and microscopic investigation demonstrate the absence of the morphological signs of the toxic effect of "Dolosan Forte®" tablets after the single intragastric administration at a dose of 6000 mg/kg to rats. The fast death of the rats receiving this drug at higher doses is possibly caused by the neurotoxic action and not by the visceral systems affection.

2. The results obtained in experiments in mice

It was registered that immediately after administration of the tablets "Dolosan Forte®" there was an acute deterioration of the animals condition: the decrease in locomotor activity, lateral position, and loss of consciousness. After 5-10 min, severe convulsions were observed both in male and female mice. Less intensive signs of intoxication were observed in animals receiving the investigated drug at a dose of 10000 mg/kg. After 20-30 min convulsions stopped, while adynamia was present. Administration of the tablets "Dolosan Forte®" at doses within the range of 11500-16000 mg/kg caused death in the first day of observation, and the dose of 18000 mg/kg led to the death within 15 min (Tab. 2).

The data listed in Tab. 2 allowed calculating of LD_{50} value for the tablets "Dolosan Forte®" administered intragastrically to mice: it equals 14393 ± 1669 mg/kg in males and 11963 ± 2023 mg/kg in females.

Table 3

LD_{50} VALUES OF "DOLOSAN FORTE®" TABLETS AND THEIR INDIVIDUAL COMPONENTS OBTAINED IN MICE USING INTRAGASTRIC ADMINISTRATION

Compound or drug	LD_{50}, mg/kg	Reference
Zirilon (2,4-dichlorobenzoic acid potassium salt)	2180 (1460 ÷ 2900)	[6]
2,4-dichlorobenzoic acid	830	[7]
Pitofenone hydrochloride	690	[14]
Fenpiverinium bromide	800	[11]
"Dolosan Forte®" tablets	14393 ± 1669 (males) 11963 ± 2023 (females)	Established in the current study

Table 4

LD_{50} VALUES OF "DOLOSAN FORTE®" TABLETS AND THEIR INDIVIDUAL COMPONENTS OBTAINED IN RATS USING INTRAGASTRIC ADMINISTRATION

Compound or drug	LD_{50}, mg/kg	Reference
Zirilon (2,4-dichlorobenzoic acid potassium salt)	No data available	
Pitofenone hydrochloride	3600	[14]
Fenpiverinium bromide	No data available	
"Dolosan Forte®" tablets	13231 ± 277 (males) 13216 ± 3379 (females)	Established in the current study

The further observations in mice that survived during 14 days shown that that they demonstrated normal activity, appearance, and appetite, responded to auditory and visual stimuli; processes of urination and defecation were normal; respiratory disorders, and seizures were not observed. There were no differences in the body weight increment in all of the experimental groups from intact control values.

Macroscopic examination did not reveal any pathological changes in animal's general state of health and well-being. The examination of the thoracic and abdominal cavities showed the normal physiological state of the internal organs, such as the thymus, heart, lungs, stomach (including mucous membrane structure), intestine and colon, liver, pancreatic gland, spleen, adrenal glands, kidneys and bladder, uterus and gonads. In the groups of male and female mice receiving the tablets "Dolosan Forte®" at doses within the range of 10000-14000 mg/kg, the relative organs weights (namely, the liver, kidneys, heart, lungs, spleen, thymus, testes) did not differ from

the intact control values, while the significant difference practically for all of the organs were seen in male mice receiving the drug at a dose of 16000 mg/kg as well as in female mice receiving the drug at a dose of 15000 mg/kg (a low number of animals in these groups due to lethality should be noted).

3. Analysis of the data obtained

Proceeding from the calculated LD_{50} values in both species, the tablets "Dolosan Forte®" refer to the V class of practically nontoxic substances (5000 mg/kg < LD_{50} < 15000 mg/kg), as listed in [2]). The obtained results show no fundamental difference between LD_{50} values in rats and mice. The species sensitivity factor, calculated as the ratio between LD_{50} of more tolerant species and LD_{50} of less tolerant species, in case of the tablets "Dolosan Forte®" does not exceed 1.1 (for males and females) demonstrating the absence of interspecies differences. Also there were no significant sex-specific differences in the investigated drug toxic effects, LD_{50} appeared to be only slightly lower in female animals (especially in mice).

It seems expedient to compare the obtained LD_{50} values with the corresponding values of the components of the investigated tablets. The available data summarized in Tab. 3 and 4 reflect the absence of toxicity increase in the combined use of 2,4-dichlorobenzoic acid potassium salt, pitofenone hydrochloride, fenpiverinium bromide.

CONCLUSIONS

1. LD_{50} for the new combined tablets "Dolosan Forte®" worked out by PJSC "Red Star" equals 13231 ± 2775 mg/kg in males and 13216 ± 3379 mg/kg in females in intragastric administration to rats; 14393 ± 1669 mg/kg in males and 11963 ± 2023 mg/kg in females in intragastric administration to mice, in either case corresponding to the V class of practically nontoxic substances (5000 mg/kg < LD_{50} < 15000 mg/kg).
2. The death of the rats and mice receiving the tablets "Dolosan Forte®" intragastrically at lethal doses can be possibly attributed to the neurotoxic action of the drug with the rapid development of convulsions.
3. Macroscopic examination demonstrated the absence of the morphological signs of the toxic effect of "Dolosan Forte®" tablets on the visceral systems of rats and mice survived after the acute intoxication (except for the increase in the relative organ weights in the groups of animals receiving doses causing high lethality rate). No pathological changes were seen at microscopic investigation of the internal organs of rats after the single intragastric administration of "Dolosan Forte®" tablets at a dose of 6000 mg/kg.
4. The data obtained showed no interspecies as well as sex-specific differences in "Dolosan Forte®" toxic effects.
5. Proceeding from the obtained results and the data in the literature, it can be concluded that combined use of zirilon (2,4-dichlorobenzoic acid potassium salt), pitofenone hydrochloride, fenpiverinium bromide does not cause the increase in their acute toxicity.

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РЕЗУЛЬТАТЫ ИЗУЧЕНИЯ ОСТРОЙ ТОКСИЧНОСТИ ТАБЛЕТОК «ДОЛОСАН ФОРТЕ®»

В настоящем исследовании определены показатели острой токсичности нового лекарственного препарата таблеток «Долосан Форте®» (в составе которых зирилон – 2,4-дихлорбензойной кислоты калиевая соль, питофенона гидрохлорид, фенпивериния бромид), разработанных ПАО «Красная Звезда». Показано, что LD_{50} этого препарата при внутрижелудочном введении составляет 3231 ± 2775 мг/кг у крыс самцов, 13216 ± 3379 мг/кг у крыс самок, 14393 ± 1669 мг/кг у мышей самцов, 11963 ± 2023 мг/кг у мышей самок, что соответствует V классу практически нетоксичных веществ (5000 мг/кг < LD_{50} < 15000 мг/кг). Гибель крыс и мышей, получавших исследуемые таблетки, вероятно, обусловлена нейротоксичностью препарата с быстрым развитием судорог. Общее состояние выживших животных было в пределах нормы в течение 14 дней. Макроскопическое обследование показало отсутствие морфологических признаков токсического действия таблеток «Долосан Форте®» на висцеральные системы выживших крыс и мышей (за исключением возрастания коэффициентов массы внутренних органов в группах животных, в которых регистрировали высокий уровень летальности). При микроскопическом изучении не выявлено патологических изменений внутренних органов крыс после однократного внутрижелудочного введения таблеток «Долосан Форте®» в дозе 6000 мг/кг. Межвидовых и межполовых отличий проявления токсических эффектов таблеток «Долосан Форте®» не наблюдалось. Полученные результаты в сочетании с данными литературы о LD_{50} компонентов «Долосана Форте®» свидетельствуют, что совместное применение зирилона (2,4-дихлорбензойной кислоты калиевой соли), питофенона гидрохлорида, фенпивериния бромида не приводит к увеличению острой токсичности.

Ключевые слова: острая токсичность; зирилон (2,4-дихлорбензойной кислоты калиевая соль); питофенона гидрохлорид; фенпивериния бромид; комбинированный лекарственный препарат; крысы; мыши

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РЕЗУЛЬТАТИ ДОСЛІДЖЕННЯ ГОСТРОЇ ТОКСИЧНОСТІ ТАБЛЕТОК «ДОЛОСАН ФОРТЕ®»

У даному дослідженні визначені показники гострої токсичності нового лікарського препарату таблеток «Долосан Форте®» (у складі яких зирілон – 2,4-дихлоробензойної кислоти калієва сіль, пітофенону гідрохлорид, фенпіверинію бромід), розроблених ПАТ «Червона Зірка». Показано, що LD_{50} цього препарату при внутрішньошлунковому введенні дорівнює 3231 ± 2775 мг/кг у щурів самців, 13216 ± 3379 мг/кг у щурів самок, 14393 ± 1669 мг/кг у мишей самців, 11963 ± 2023 мг/кг у мишей самиць, що відповідає V класу практично нетоксичних речовин (5000 мг/кг < LD_{50} < 15000 мг/кг). Загибель щурів та мишей, які одержували досліджувані таблетки, ймовірно, спричинена нейротоксичністю препарату зі швидким розвитком судом. Загальний стан тварин, що вижили, був у межах норми впродовж 14 днів. Макроскопічне обстеження показало відсутність морфологічних ознак токсичної дії таблеток «Долосан Форте®» на вісцеральні системи щурів та мишей, що вижили (за винятком збільшення коефіцієнтів маси внутрішніх органів у групах тварин, у яких реєстрували значний рівень летальності). При мікроскопічному дослідженні не виявлено патологічних змін внутрішніх органів щурів після одноразового внутрішньошлункового введення таблеток «Долосан Форте®» у дозі 6000 мг/кг. Міжвидових або міжстатевих відмінностей у виявленні токсичних ефектів таблеток «Долосан Форте®» не спостерігали. Отримані результати у поєднанні з даними літератури щодо LD_{50} компонентів «Долосану Форте®» свідчать, що поєднане застосування зирілону (2,4-дихлоробензойної кислоти калієвої солі), пітофенону гідрохлориду, фенпіверинію броміду не призводить до зростання гострої токсичності.

Ключові слова: гостра токсичність; зирілон (2,4-дихлоробензойної кислоти калієва сіль; пітофенону гідрохлорид; фенпіверинію бромід; комбінований лікарський препарат; щури; миші

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