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STUDY OF ANTHELMINTIC ACTIVITY AND ACUTE TOXICITY OF MEDICINE OF COMBINED COMPOSITION

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Meta. Метою роботи є вивчення антигельмінтної активності та гострої токсичності препарату, що містить альбендазол та празиквантел у співвідношенні (1: 4) щодо збудників аскаридозу у свиней, токсокарозу та дипілідіозу у собак. Ці патогени належать до класу нематодозів (аскаридозу, токсокароз) та цестодозів (дипілідіоз).

Матеріали та методи. Дослідження проводили у копроскопічній лабораторії кафедри паразитології ХДЗВА за стандартизованим методом Фюллеборна і «Способом кількісного визначення яєць гельмінтів» (патент № 9265). Зразки для дослідження на собаках були отримані в КП "Центр поводження з тваринами". Для вивчення ступеня токсичності запропонованої комбінації альбендазолу і празиквантелу у посію відбирався проби крові перед прийомом препарату та через 24 год і 72 год після початку лікування для проведення морфологічних і біохімічних досліджень.

Результати. Отримані результати свідчать про наявність антигельмінтної активності досліджуваного препарату по відношенню до збудників аскаридозу, токсокарозу та дипілідіозу. Показники гематологічних досліджень у свиней більш вільних від кишкових гельмінтів до та через 24 і 72 год після прийому препарату знаходились у межах фізіологічної норми. Результати клінічного обстеження тварин обох дослідних груп показали, що поведінка тварин не змінилася (природня), прийом корму і води в нормі, видимі слизові оболонки – блідо-рожевого кольору, шкіра еластична.

Висновки. Таким чином, запропонований препарат демонструє високий рівень антигельмінтної активності щодо збудників аскаридозу, токсокарозу та дипілідіозу. Ступінь його токсичності відповідає показникам "малотоксичний". Отримані результати вказують на доцільність подальших досліджень.

Ключові слова: антигельмінтні препарати, альбендазол, празиквантел, нематодоз, цестодоз, фармакологічні дослідження
1. Introduction
The problem of parasitic diseases in general and helminth infestations in particular, has a sharp social significance in the modern world. Active dissemination of pathogens, including those that are not typical for certain regions and countries, neglect of the rules of personal hygiene and problems in the field of health insurance lead to an increase in incidence of diseases among the population.

2. Formulation of the problem in a general way, the relevance of the theme and its connection with important scientific and practical issues
According to the World Health Organization, about 16 million people die each year from diseases of infectious and parasitic etiology [1]. In this case, children are the most vulnerable to this group of diseases [2, 3]. In children, helminthiasis of the digestive system account for 92.3 % of enterobiasis cases, 71.1 % of ascariasis cases, 61.5 % of trichocephalosis cases and 66.2 % of toxocarosis cases [2, 4].

According to the World Bank, the economic losses from helminthiasis of the digestive system occupy the fourth place in the overall structure of diseases and injuries of the planet’s population [5].

3. Analysis of recent studies and publications in which a solution of the problem and which draws on the author
Now, the existing range of medicines for the treatment of helminthiasis of the digestive system, represented on the pharmaceutical market of Ukraine, is presented by medicines of foreign production that are quite expensive [6]. The main side effects of such medicines are nausea, vomiting, pain in epigastrium, diarrhea, allergy, dizziness, headache, neurological disorders, impaired liver function, increased body temperature and drowsiness [7].

4. Allocation of unsolved parts of the general problem, which is dedicated to the article
As the range of anthelmintic medicines is represented by monodrugs [8], whereas in most cases it is recommended to combine several active pharmaceutical ingredients while etiotropic treatment to achieve a quick and complete elimination of pathogens from the patient’s body [9], there were conducted the relevant studies on combination of albendazole and praziquantel in different ratios.

5. Formulation of goals (tasks) of article
We proposed the complex anthelmintic drug with albendazole and praziquantel in ratio (1:4) as active substances. The purpose of our work was to study the anthelmintic activity of this drug and study the degree of its acute toxicity.

6. Statement of the basic material of the study (methods and objects) with the justification of the results
Materials and methods. Investigation was conducted in 2 stages:
1 – in the period from April 16 to April 29, 2018 – the experiment on pigs (n = 12) was conducted in SPC Kharkov State Zooveterinary Academy (KSZVA);
2 – in the period from April 18 to May 5, 2018 – the experiment on dogs (n = 12) was conducted in the CP “Center for Animal Welfare”.

Each animal species was divided into 2 groups: experimental (n = 6) and control (n=6). The animals of the experimental group were given the studied drug in the following doses:
- for pigs: 10 mg/kg of body weight on albendazole and 40 mg/kg of body weight on praziquantel;
- for dogs: 20 mg/kg of body weight on albendazole and 80 mg/kg of body weight on praziquantel.

The foregoing effective doses of the drug correspond to the generally accepted recommended doses of albendazole for anthelmintic therapy in pigs and dogs, respectively [10, 11].

Animals in the control group did not receive the drug.

The anthelmintic activity of the drugs was studied in relation to pathogens of ascariasis in pigs, toxocarosis and dipylidiosis in dogs. These pathogens belong to the class of nematodoses (ascariasis, toxocarosis) and cestodoses (dipylidiosis), and are those often found among both selected species of animals and in humans. Infection of animals with helminthes occurred naturally in 3 months. The presence of pathogens in the body of animals was determined by a coprosopic method, in accordance with generally accepted rules for the diagnosis of each type of pathogen.

The studied drug was administered in the therapeutic regime to the experimental groups of animals orally for 7 days.

The effectiveness of the treatment was evaluated by the coprosopic method of the diagnosis of each type of pathogen. The proper studies were carried out in the coprosopic laboratory of the parasitology department of Kharkov State Zooveterinary Academy by the standardized method of Füllbourne and the “Method for the quantitative determination of helmith eggs” (patent No. 9265) [12].

The degree of toxicity and the overall effect of the studied drug was evaluated on the basis of clinical examination (inspection) in both experimental groups and the morphological and biochemical studies of the blood samples of piglets (blood samples were taken before the beginning of the treatment and after 24 and 72 hours after the first intake of the drug). Number of erythrocytes and leucocytes was determined in heparin stabilized blood of piglets using the Goryaev counting chamber; hemoglobin content was determined by hemoglobin-cyanide method; the hematocrit was determined by the method of microcentrifugation according to Shiklar. The leukogram was deduced by counting of individual white blood cells in the smears painted according to Romanovsky-Gimza.

The following parameters were determined in blood serum: urea level – by reaction with diacetyl-miooxime, creatinine – by Jaffé’s colour reaction, activity of aspartate aminotransferase (AsAT) and alanine aminotransferase (AIA) – by Reitman-Frenkel method using reagent sets of PJSC “Reagent” (Ukraine) produc-
tion according to the methods described by Kamyschnikov V. S. [13].

Data processing was carried out using methods of statistical analysis [14, 15].

7. Findings from the research and prospects of further development of this area

Results and discussion. Results of coproscopic studies in pigs are presented in Table 1.

As seen from Table 1, the administration of the studied drug provides complete elimination of ascarides from the body of piglets within 7 days. A follow-up study a week after discontinuation of therapy showed no recurrence of the disease. These results indicate the absolute effectiveness of the studied drug in relation to ascariasis pathogens. The results of hematological studies in the blood of piglets are presented in Table 2.

### Table 1

| Animal no. | Before treatment | on the 7th | on the 14th | Effectiveness, % |
|------------|-----------------|-----------|------------|-----------------|
|            | II. eggs in 1 g of feces |            |            |                 |
| Experimental group |                   |            |            |                 |
| 1          | 13.3            | –         | –          | –               |
| 2          | 15.7            | –         | –          | –               |
| 3          | 12.7            | –         | –          | –               |
| 4          | 11.0            | –         | –          | –               |
| 5          | 18.3            | –         | –          | –               |
| 6          | 15.3            | –         | –          | –               |
| M±m       | 14.4±1.1        | –         | –          | –               |

Control group

|            | –         | –         | 12.3       |
|------------|-----------|-----------|------------|
| 1          | 12.3      | 13.0      | 12.3       |
| 2          | 15.3      | 15.0      | 15.0       |
| 3          | 10.3      | 13.0      | 13.0       |
| 4          | 9.0       | 9.7       | 10.0       |
| 5          | 12.0      | 11.7      | 12.3       |
| 6          | 11.7      | 13.0      | 13.0       |
| M±m       | 11.8±0.9  | 12.1±0.8  | 12.1±0.8   |

Note: \( p \leq 0.05 \)

### Table 2

| Sampling time Indicators | No.1 Before administration of the drug | 24 h | 72 h | No.2 Before administration of the drug | 24 h | 72 h | No.3 Before administration of the drug | 24 h | 72 h | No.4 Before administration of the drug | 24 h | 72 h |
|-------------------------|---------------------------------------|------|------|---------------------------------------|------|------|---------------------------------------|------|------|---------------------------------------|------|------|
| Morphological study     |                                       |      |      |                                       |      |      |                                       |      |      |                                       |      |      |
| Erythro-cytes, g/l     | 6.7                                   | 7.3  | 6.3  | 6.6                                   | 7.5  | 6.7  | 5.5                                   | 6.0  | 7.0  | 6.8                                   | 6.2  | 6.3  |
| Hemo-globin, g/l       | 133.7                                 | 140.8| 143.4| 129.8                                 | 144.5| 134.5| 137.1                                 | 138.6| 135.3| 129.3                                 | 137.1| 135.3|
| Leukocytes, t/l        | 15.8                                  | 20.6 | 14.2 | 18.5                                  | 17.3 | 17.6 | 12.0                                  | 15.5 | 13.5 | 16.0                                  | 10.2 | 17.2 |
| Hematocrit, %          | 36.4                                  | 42   | 34.5 | 38.8                                  | 30.6 | 34.0 | 36.2                                  | 38.2 | 38.4 | 38.3                                  | 36.1 | 39.1 |
| Leukoformula (%)       |                                       |      |      |                                       |      |      |                                       |      |      |                                       |      |      |
| Young                  | 2                                     | 0    | 0    | 0                                     | 0    | 0    | 0                                     | 1    | 1    | 0                                     | 0    | 0    |
| Stab                   | 13                                    | 7    | 5    | 8                                     | 5    | 5    | 8                                     | 6    | 9    | 6                                     | 8    | 5    |
| Segmented              | 49                                    | 39   | 37   | 49                                    | 30   | 40   | 40                                    | 31   | 43   | 46                                    | 50   | 48   |
| Eosinophils            | 2                                     | 2    | 1    | 1                                     | 0    | 1    | 1                                     | 6    | 2    | 2                                     | 1    | 3    |
| Monocytes              | 10                                    | 4    | 8    | 5                                     | 10   | 2   | 2                                     | 10   | 7   | 10                                    |       |      |
| Basophils              | 0                                     | 0    | 0    | 2                                     | 0    | 0    | 0                                     | 0    | 0    | 0                                     | 0    | 0    |
| Lymphocytes            | 24                                    | 48   | 47   | 37                                    | 57   | 51  | 41                                    | 55   | 25   | 35                                    | 34   | 34   |
| Biochemical study      |                                       |      |      |                                       |      |      |                                       |      |      |                                       |      |      |
| Creatinine, μmol/l     | 73.3                                  | 80.4 | 78.4 | 83.5                                  | 75.3 | 86.5 | 75.3                                  | 72.3 | 82.4 | 95.7                                  | 83.0 | 78.4 |
| Urea, mmol/l           | 3.13                                  | 2.97 | 2.16 | 2.94                                  | 1.87 | 1.48 | 1.68                                  | 1.72 | 3.42 | 2.35                                  | 2.39 | 3.16 |
| AlAT, un/l             | 52.5                                  | 47.4 | 44.8 | 51.5                                  | 50.9 | 40.2 | 42.9                                  | 47.4 | 46.7 | 39.2                                  | 44.8 | 54.7 |
| AcAT, un/l             | 47.8                                  | 73.0 | 54.5 | 56.1                                  | 86.1 | 55.0 | 53.2                                  | 52.1 | 46.5 | 57.9                                  | 56.4 | 61.2 |

Note: \( p \leq 0.05 \)
As seen from Table 2, indicators of hematological studies in pigs free from intestinal helminthes before 24 h and 72 h after drug administration were within the limits of the physiological norm. Drug intake does not lead to violations of morphological indicators, changes of leukoformula and biochemical indicators of blood.

The further clinical examination (inspection) of animals in both experimental groups showed that the behavior of the animals remained unchanged (natural), the intake of food and water was in the normal, visible mucous membranes were pale pink color, the skin – integral, no damage, elastic.

Taking into account the results obtained, it can be concluded that the acute toxicity of the studied drug corresponds to the degree of toxicity “low toxic”, which is similar to the result of pure albedazole [16].

Results of coproscopic studies in dogs are presented in Table 3.

Effectiveness of the studied combined anthelmintic drug when toxocarosis and dipylidiosis in dogs (n=12)

| Animal no. | II, eggs in 1 g of feces | Before treatment | on the 7th day after treatment | on the 14th day after treatment | E, % | E, % |
|------------|--------------------------|-----------------|------------------------------|-------------------------------|------|------|
|            |                          | T               | D                            | T                             | D    | D    |
| Experimental group | 1 | 24.3 | 15.7 | – | – | – | – | – |
|              | 2 | 15.3 | 16.7 | – | – | – | – | – |
|              | 3 | 18.0 | 13.0 | – | – | – | – | – |
|              | 4 | 17.7 | 13.7 | – | – | – | – | – |
|              | 5 | 29.0 | 14.7 | – | – | – | – | – |
|              | 6 | 27.0 | 29.3 | – | – | – | – | – |
| M±m         | 21.9±2.3 | 17.2±2.5 | – | – | – | – | – |
| Control group | 1 | 12.7 | 20.0 | 12.0 | 18.3 | 13.3 | 19.7 | 100 |
|              | 2 | 15.3 | 18.3 | 16.7 | 16.7 | 16.3 | 19.0 | – |
|              | 3 | 18.3 | 17.3 | 20.0 | 19.0 | 19.3 | 18.3 | – |
|              | 4 | 22.3 | 21.0 | 21.0 | 21.7 | 23.0 | 23.0 | – |
|              | 5 | 20.0 | 23.7 | 19.3 | 25.3 | 21.3 | 24.3 | – |
|              | 6 | 15.3 | 18.0 | 16.3 | 16.3 | 15.7 | 17.3 | – |
| M±m         | 17.3±1.4 | 19.7±1.0 | 17.6±1.3 | 19.6±1.4 | 18.2±1.5 | 20.3±1.1 | – | – |

Note: T – toxocarosis, D – dipylidiosis, E – effectiveness; p≤0.05

Results of the studied drug administration in dogs showed that the combination of albendazole and praziquantel provides complete elimination of toxocarosis and dipylidiosis pathogens from the body of dogs within 7 days. As in the case of ascariasis described above, the further investigation a week after therapy discontinuation showed no recurrence of the disease. These results indicate the absolute effectiveness of the studied drug in relation to toxocarosis and dipylidiosis pathogens.

**8. Conclusion**

The studied drug, which contains the mixture of albendazole and praziquantel in the ratio (1:4), proved to be effective in relation to pathogens of ascariasis in pigs, toxocarosis and dipylidiosis in dogs. Drug intake during 7 days provides the complete elimination of helmintases with no recurrence of the disease.

Hematological studies in pigs showed that the morphological indicators, leukoformula and biochemical indicators of blood remain in the limits of the physiological norm 24 h and 72 h after drug intake. According to the results of a clinical examination (inspection) of both pigs and dogs at the beginning and during the experiment it was established that the behavior of the animals remained unchanged (natural), the intake of food and water was in the normal, visible mucous membranes were pale pink color, the skin – integral, no damage, elastic. The obtained results allow concluding that the acute toxicity of the studied drug corresponds to the degree of toxicity “low toxic”, which is similar to the result of pure albedazole.

On the basis of the obtained results it can be concluded that the proposed drug has the proved anthelmintic activity with “low toxic” of degree of toxicity. Therefore, it is expedient to carry out the further research and to introduce the proposed drug into industrial production in order to expand the existing range of anthelmintic medicines represented on Ukrainian pharmaceutical market.

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