Increasing the biological activity of benzimidazoles based on the supramolecular nanoscale delivery system with licorice extract against helminthiasis

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Abstract: This paper considers the effect of mechanochemical technology on the anthelmintic efficacy of a solid dispersion (SD) of albendazole (ABZ) with licorice extract (LE) / SDALE /, which is considered as a means of targeted delivery. SDALE tests were carried out on white mice experimentally infected with Trichinella spiralis and Hymenolepis nana and on sheep naturally infected with gastrointestinal strongylates and Moniezia expansa cestodes. For each helminthiasis, animals of different groups of 9–12 in each were orally administered once a dose of SDALE of 2.0 mg/kg of active substance (AS) in the form of 10–20% powders and 1 and 2% suspensions in comparison with the basic drug - a substance of albendazole at the dose of 2.0 mg/kg. SDALE is prepared by joint milling of similar components (ABZ and LE) in grinders with adjustable immersion energy (module, time, weight ratio of components, drum rotation speed) for 2 hours. Animals of the control groups did not receive the drug. The activity of the drugs was determined according to the data of coproovoscopie examination of sheep by flotation method before and 15 days after treatment. SDALE at the dose of 2.0 mg/kg of AS in the form of 10 and 20% powders and 1 and 2% suspensions showed respectively 91.4; 89.3; 87.2 and 92.3% efficacy against Nematodirus spp. and 90.7; 88.4; 87.4 and 91.2% activity against other types of Strongylata when receiving a 23.2 and 22.7% effect of the base drug - ABZ.

1. Introduction
Helminth infections of animals are widespread in the Russian Federation and other countries of the world and cause significant economic damage due to the death of animals and reduced productivity [1, 2]. The most widely used drug for treating animals is albendazole (ABZ), which has a wide spectrum of anthelmintic activity at a dose of 5–10 mg/kg [3-5]. The drug is effective for helminths, including nematodosis, cestodosis and trematodosis. ABZ at a dose of 400 mg × 3 is also effective for human nematodosis [3]. It has been successfully tested for the treatment of human cysticercosis and echinococcosis [6, 7]. However, the drug has disadvantages, namely the presence of embryotrophic
activity in an increased dose and poor solubility in water, i.e., low permeability, which leads to poor bioavailability and increased doses, especially with cestodosis and trematodosis. In this regard, it is of interest to study ways to increase its efficacy and reduce the therapeutic dose due to targeted delivery of the drug using carriers to the active centers of the corresponding receptors. The most commonly used drug delivery vehicles are cyclodextrins, with the help of which supramolecular systems are formed [8], as well as polysaccharides, liposomes, micelles and nanoscale inorganic particles [9]. There are reports of the preparation of microcrystalline compositions of albendazole based on chitosan, cellulose derivatives and poloxamer as a surfactant [10]. One of the technologies of targeted delivery is the mechanochemical modification of solid medicinal substances and excipients. Under the influence of pressure and shear deformations in impact-abrasive mills, the crystal structure of substances can be disordered to complete amorphization and polymorphic transitions and chemical reactions occur with the formation of complexes or micelles with increased solubility [11, 12].

In previous years, the supramolecular complex of ABZ with arabinogalactan was tested, obtained by mechanochemical technology, which has increased solubility in water and better efficiency [13]. However, this drug was not used because of the high cost of arabinogalactan. In this regard, SDA with LE (hereinafter referred to as SDALE), which is available for use, since it is not expensive.

Considering the above, the goal of the current research was to test the SDALE prepared by mechanochemical technology in impact-grinding grinders. The purpose of the study is to evaluate the anthelmintic efficacy of SDALE.

2. Materials and methods
SDALE was obtained by mechanochemical technology with the addition of a VM-1 type roller mill with a volume of 1000 ml of 50 g of LE and 10 g of ABZ into a metal drum with the addition of 1800 g of metal balls with a diameter of 25 mm. The drum was mounted on rolls and the mixture was processed for 2 hours while the drum was rotating at a speed of 70 rpm. The obtained product SDALE in the ratio ABZ: LE = 1: 20 in the form of a light brown granular powder was unloaded from the drum (preparation No. 1). Similarly, the product SDALE was obtained in the ratio of ABZ: LE = 1: 9 (preparation No. 2) from 5 g of ABZ and 50 g of LE. The anthelmintic properties of these drugs were further studied.

In another grinder, the LE-101 roller mill, 9 g of the substance of albendazole and 81 g of LE were loaded into a metal drum (800 ml), and after preliminary mixing, 1600 g of balls were added. Machining was performed at a drum rotation speed of 70-80 rpm for 3 hours. The obtained product SDALE in the ratio ABZ: LE = 1: 9 in the form of a brown loose powder was unloaded from the drum (preparation No. 3). Similarly, the product SDALE was obtained in the ratio of ABZ: LE = 1: 4 (preparation No. 4) from 18 g of ABZ and 72 g of LE.

Nematodicidal activity of SDALE was studied on a laboratory model of trichinellosis in 70 white mice experimentally infected by *T. spiralis* at a dose of 200 larvae per animal. On the third day after infection, the mice of the experimental groups (10 animals each) were orally administered once drugs No. 1 and No. 2 in the form of a powder and in the form of a 1 and 2% suspension at a dose of 2.0 mg/kg of AS. Mice of the 5th and 6th groups received respectively a physical mixture of ABZ with LE and the basic drug, the substance ABZ, at a dose of 2.0 mg/kg. Animals of the control group received distilled water. Animals were killed by decapitation on the second day after drug administration. The efficacy of the drugs was evaluated by the results of intestines necropsy, taking scrapings, digesting in a solution of artificial gastric juice and counting under a binocular magnifier. The efficacy of the drugs was counted according to the type of “control test” with the calculation of the average number of detected nematodes and intense of efficacy.

The cestodocidal activity of SDALE was evaluated on 70 white mice experimentally infected with *H. nana* at a dose of 200 infective eggs per animal. The tested drugs in the same dose were administered to mice of various groups in the same way as with trichinosis [14].
On the fourth day after administration, the mice were killed, after intestines necropsy the detected cestodes were counted. Accounting for the efficacy of the drugs was carried out according to the type of “control test” with the calculation of the average number of cestodes and intense of efficacy.

The anthelmintic properties of SDALE were studied in the sheep farm of Izmailov LLC in the Krasnoarmeysky district of the Samara region, which is ineffective for helmint infections. The study of drugs was carried out in August – September 2019 during the period of maximum infection of animals. In the experiments, 180 young sheep of Adelbaev breed with a body weight of 22 to 39 kg were used, including sheep naturally infected with nematodirusis (70) and other types of gastrointestinal strongylates (70) and moniesia (70). For each helmint animals were divided by the principle of analogues into 7 equivalent groups of 9–11 sheep each. Sheep of the first and second groups were given the drugs No. 2 and No. 1, respectively, in the form of a 10 and 20% powder once orally at a dose of 2.0 mg/kg in of AS. Sheep of the third and fourth groups received SDALE in the form of, respectively, 1 and 2% suspension. Animals of the fifth group received a physical mixture of ABZ with LE without mechanochemical treatment at a dose of 2.0 mg/kg of AS. Sheep of the sixth group was given the basic drug - the substance ABZ, on the basis of which SDALE was prepared, also at a dose of 2.0 mg/kg. Animals of the control group did not receive the drug.

The efficacy of SDALE was evaluated according to the results of coproovoscopic examination by flotation method before and 15–16 days after drug administration [15]. Accounting for the efficacy of drugs was carried out according to the type of “control test” with the calculation of the average number of detected eggs of nematodes [16]. The obtained results were processed statistically using the computer program Microsoft Excel 2003-2007.

3. The discussion of the results

At studying the solubility in water of the substance ABZ and its solid dispersions, as well as suspensions based on solid dispersions, a significant change in this indicator was established. The solubility data of ABZ are shown in table 1.

| Option, its composition, machining time m/o and ABZ content (%) | Solubility, absolute, mg/l | increase, times |
|---------------------------------------------------------------|-----------------------------|----------------|
| ABZ – basic substance (97.0)                                  | 1.0                         | –              |
| Drug No. 3 (ABZ: LE = 1: 9), m/o 3 hours (10.0)               | 16.5                        | 17             |
| Drug No. 4 (ABZ: LE = 1: 4), m/o 3 hours (20.0)               | 12.5                        | 13             |
| 10% suspension from drug No. 3 (10.0)                         | 69.1                        | 69             |
| 20% suspension from drug No. 4 (20.0)                         | 69.0                        | 69             |
| Physical mixture composition ABZ: LE = 1: 9 (10.0)            | 2.6                         | 3              |

The drug No. 3 had a 17 times increased solubility after three hours of machining. With an increase in the share of ABZ in SDALE from 10% (drug No. 3) to 20% (drug No. 4), the solubility decreased to 13 times. The greatest increase in solubility was observed while producing the SDALE in the form of a suspension. In a physical mixture, the solubility of ABZ increases only 3 times.

The results of a test of SDALE at a dose of 2.0 mg/kg of AS on a laboratory model against T. spiralis are shown in Table 2 and indicate a significant increase in the activity of drugs No. 1 and No. 2. So 96.3–96.9% efficacy of SDALE at a dose of 2 ml/kg of AS was received. There was no significant difference in the efficacies of SDALE in the form of 1 and 2% suspensions and 10 and 20% powders (P <0.05). A physical mixture of ABZ and LE in the ratio of 1:10 at a dose of 2 mg/kg of AS showed a 20.4% effect, which is 4.8 times lower than SDALE in the same dose. The basic drug ABZ at a dose of 2.0 mg/kg showed 17.6% activity. In animals of the control group, an average of 78.3 ± 7.4 examples of T. spiralis were found.

The study of SDALE on white mice experimentally infected with H. nana also showed a significant (4.6-fold) increase in the efficacy of SDALE compared to the activity of a physical mixture with the
basic drug. The efficiency of SDALE at a dose of 2.0 mg/kg of AS in the form of 10 and 20% powder was 89.2 and 88.2%, respectively, and in the form of 1 and 2% suspensions, 89.2 and 87.11%. The activity of the physical mixture of ABZ and LE at a dose of 2.0 mg/kg of AS was 19.3%, and that of the base drug, ABZ substances, was 13.9%. Thus, a significant increase in the anthelmintic activity of SDALE has been established with trichinellosis and hymenolepiosis, which is due to an increase in the solubility and bioavailability of SDALE.

**Table 2.** The efficacy of SDALE at a dose of 2.0 mg/kg of AS against *Trichinella spiralis* and *Hymenolepis nana* (n = 10 animal units).

| The drug, the ratio of components, the concentration of ABZ (%), machining time (m/o) | The average number of helminths | Efficiency% |
|---|---|---|
| *Trichinella spiralis* | | |
| Drug No. 2 (ABZ: ES = 1: 10) (9.1) 3 hours m/o | 2.4±0.3 | 96.94 |
| Drug No. 1 (ABZ: ES = 1: 20) (4.8) 3 hours m/o | 2.7±0.3 | 96.56 |
| 1% suspension of the drug No. 2 | 2.6±0.3 | 96.68 |
| 2% suspension from drug No. 1 | 2.9±0.4 | 96.30 |
| Physical mixture ABZ: LE (1: 10) | 62.3±5.7 | 20.44 |
| ABZ base 99% powder | 64.5±6.6 | 17.63 |
| Control | 78.3±7.4 | – |
| *Hymenolepis nana* | | |
| Drug No. 2 (ABZ: ES = 1: 10) (9.1) 3 hours m/o | 1.0±0.3 | 89.25 |
| Drug No. 1 (ABZ: ES = 1: 20) (4.8) 3 hours m/o | 1.1±0.3 | 88.18 |
| 1% suspension of the drug No. 2 | 1.0±0.3 | 89.25 |
| 2% suspension from drug No. 1 | 1.2±0.2 | 87.10 |
| Physical mixture ABZ: LE (1: 10) | 7.5±1.3 | 19.36 |
| ABZ basic 99% powder | 8.0±1.3 | 13.98 |
| Control | 9.3±1.4 | – |

The obtained results of the SDALE test on sheep are shown in Table 3 and indicate a different degree of efficacy of drugs in different forms and ratios against various types of helminths.

SDALE in the ratio of 1:10 and 1:5 (drugs No. 2 and No. 1, respectively) in the form of a powder showed 91.7 and 90.4% respectively against *Nematodirus spp.* according to the results of coproscopic examination. SDALE in a ratio of 1:10 and 1:5 (drugs No. 2 and No. 1, respectively) in the form of a 1 and 2% suspension showed 91.0 and 90.1% effects, respectively, against sheep nematodiosis. The efficacy of the basic drug - substance ABZ was at a dose of 2.0 mg/kg 12.94 %, and the physical mixture of ABZ with LE in the same dose was 11.19%. The infection of sheep in the control group did not change significantly during the experiment (P ≥ 0.05).

In other strongylatosis of the digestive tract of sheep, SDALE in the ratio of 1:10 and 1:5 (drugs No. 2 and No. 1, respectively) in the form of 10 and 20% powders showed 92.4 and 89.5% efficiency, respectively, and the same drugs in the form of a 1 and 2% suspension showed 90.9 and 91.4% activity, respectively. A physical mixture of ABZ and LE in a ratio of 1:10 showed 21.2% efficiency in the same dose. The substance ABZ in a dose of 2.0 mg/kg showed only 18.9% effect. The number of eggs of strongylates per gram of feces at the beginning and end of the experiment did not change significantly and amounted to 130.2 ± 8.1 and 136.0 ± 8.1, respectively.

There is no significant difference in increasing the efficacy of SDALE in the form of suspension and powder, since the latter was administered orally to the sheep also in the form of a suspension with water.

The results of the SDALE examination against sheep moniesiosis caused by *Moniezia expansa* indicate a 100% efficacy of the drug No. 2 and its 1% suspension in a dose of 2.0 mg/kg of AS. High (98.7 and 99.0%) efficacy against *M. expansa* was also shown by drug No. 1 and its 2% suspension at a dose of 2.0 mg/kg of AS. The physical mixture of ABZ and LE in a ratio of 1:10 and the basic drug...
ABZ at this dose were ineffective in case of moniesiosis. In 1 g of feces of sheep of the control group, an average of 175.2 ± 7.5 examples of *M. expansa* were found.

4. Conclusions

The possibility of mechanochemical modification of the substance of albendazole with licorice extract has been shown. In this case, solid dispersions of various ABZ compositions with LE were obtained, and had an increased solubility in water in comparison with the substance of ABZ itself and its physical mixture with LE. Optimal formulations were tested for anthelmintic activity on laboratory models and in field conditions on sheep.

In experiments on sheep naturally infected with nematodirusis and other species of strongylates of the digestive tract and moniesia, it was shown that SDALE at a dose of 2.0 mg/kg of AS showed 90.1–91.7% efficacy against *Nematodirus spp.* and 89.5–92.4% efficacy against other types of gastrointestinal strongylates and 98.6–100% efficacy against *M. expansa*, which is in 4–5 times higher than ABZ activity.

**Table 3.** The efficacy of SDA with LE in a dose of 2.0 mg/kg of AS against helminthiasis of sheep.

| The drug, the ratio of components, the concentration of ABZ (%), the time of machining (m/o) | Infected before experiment, number of animals | Free from infection, number of animals | The average number of nematode eggs per gram of feces, specimen Before experiment | Decrease in the number of helminth eggs in feces, % |
|---|---|---|---|---|---|
| *Nematodirosis* | | | | | |
| Drug No. 2 (ABZ: LE = 1: 10) (9.1) 3 hours m/o | 11 | 10 | 121.7±7.7 | 10.4±2.3 | 91.70 |
| Drug No. 1 (ABZ: LE = 1: 20) (4.8) 3 hours m/o | 10 | 7 | 120.4±7.2 | 12.0±2.5 | 90.42 |
| 1% suspension of the drug No. 2 | 9 | 7 | 119.3±6.9 | 11.2±2.6 | 91.06 |
| 2% suspension from drug No. 1 | 10 | 8 | 121.4±7.3 | 12.4±3.0 | 90.10 |
| Physical mixture ABZ: LE (1: 10) | 10 | 0 | 123.6±8.0 | 11.2±7.0 | 11.19 |
| ABZ base 99% powder | 9 | 0 | 122.0±6.9 | 109.0±7.2 | 12.94 |
| Control | 10 | 0 | 118.6±6.8 | 125.2±7.7 | – |
| *Other strongylates of the digestive tract* | | | | | |
| Drug No. 2 (ABZ: ES = 1: 10) (9.1) 3 hours m/o | 10 | 9 | 132.4±8.1 | 10.3±2.4 | 92.43 |
| Drug No. 1 (ABZ: ES = 1: 20) (4.8) 3 hours m/o | 11 | 9 | 130.6±7.7 | 14.2±3.0 | 89.56 |
| 1% suspension of the drug No. 2 | 9 | 8 | 131.2±7.8 | 12.3±2.5 | 90.96 |
| 2% suspension from drug No. 1 | 10 | 6 | 133.4±8.0 | 11.6±2.7 | 91.47 |
| Physical mixture ABZ: LE (1: 10) | 9 | 0 | 132.0±7.8 | 107.0±7.9 | 21.23 |
| ABZ basic 99% powder | 9 | 0 | 131.3±8.1 | 110.2±8.0 | 18.98 |
| Control | 9 | 0 | 130.2±7.9 | 136.0±8.1 | – |

**Moniesiosis**

| The drug, the ratio of components, the concentration of ABZ (%), the time of machining (m/o) | Infected before experiment, number of animals | Free from infection, number of animals | The average number of nematode eggs per gram of feces, specimen Before experiment | Decrease in the number of helminth eggs in feces, % |
|---|---|---|---|---|---|
| Drug No. 2 (ABZ: LE = 1: 10) (9.1) 3 hours m/o | 10 | 10 | 172.3±6.8 | 0 | 100 |
| Drug No. 1 (ABZ: LE = 1: 20) (4.8) 3 hours m/o | 9 | 8 | 169.4±7.2 | 2.3±0.3 | 98.69 |
| 1% suspension of the drug No. 2 | 11 | 11 | 171.6±6.9 | 0 | 100 |
2% suspension from drug No. 1 | 9 | 8 | 170.2±7.1 | 1.7±0.2 | 99.03
---|---|---|---|---|---
Physical mixture ABZ: LE (1: 10) | 10 | 0 | 174.3±7.5 | 156.3±6.3 | 10.80
ABZ base 99% powder | 9 | 0 | 168.9±7.2 | 157.6±6.8 | 10.05
Control | 9 | 0 | 171.4±7.1 | 175.2±7.5 | –

**Acknowledgements**

This work was supported by the Ministry of Science and Higher Education of the Russian Federation.

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