

ORIGINAL ARTICLE

Influence of mechanochemical technology on anthelmintic efficacy of the supramolecular complex of fenbendazole with polyvinylpyrrolidone

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ABSTRACT

Objective: The purpose of our research was to evaluate the effect of mechanochemical technology on the efficacy of supramolecular complex of fenbendazole (SMCF) with polyvinylpyrrolidone (PVP) polymer against some helminthosis of animals.

Materials and Methods: The SMCF samples with PVP were synthesized using a solid-state mechanochemical technology in activators of impact-abrading type and their physicochemical properties were analyzed. The efficacy of SMCF was studied on the laboratory model of *Hymenolepis nana* and *Trichinella spiralis* infection of mice and helminthosis of sheep.

Results: In the trials conducted on laboratory models, the supramolecular complex showed 93.94% and 98.56% efficacy at the dose of 1 mg/kg of body weight (b/w), while the substance of fenbendazole showed 7.97% and 8.33% efficacy at the same dose. A high efficacy (>94%) of the SMCF was revealed at the dose of 2.0 mg/kg of b/w at oral administration against nematodes in naturally infected sheep by the results of the fecal examination, while the substance of fenbendazole was active at the dose of 5.0 mg/kg at single oral administration. Moreover, the SMCF demonstrated 97.37% efficacy at the dose of 2 mg/kg against *Moniezia* spp. infection of sheep. Physicochemical studies confirmed the increase in solubility of the complex, reducing of particle sizes, amorphization of fenbendazole substance, and incorporating it with micelles of PVP.

Conclusion: According to the results, supramolecular complex of fenbendazole with PVP was more active than the basic substance of fenbendazole and its anthelmintic properties were expanded.

ARTICLE HISTORY

Received December 17, 2018

Revised January 10, 2019

Accepted January 12, 2019

Published February 25, 2019

KEYWORDS

Efficacy; fenbendazole; polyvinylpyrrolidone polymer; supramolecular complex; mechanochemistry.



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Introduction

Helminthosis of animals is widespread and about 40%–90% of sheep and cattle are infected by helminths in some regions of Russia [1,2]. Helminthosis causes huge economic damage because of significant reduction in the

growth and development of young animals, as well as a reduction in the quantity and quality of products. It was found that the body weight gain of the calf infected with *Dictyocaulus viviparus* and gastrointestinal strongylates is reduced to 34–35 kg per year [3].

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How to cite: Arkhipov IA, Khalikov SS, Sadov KM, Dushkin AV, Meteleva ES, Varlamova AI, et al. Influence of mechanochemical technology on anthelmintic efficacy of the supramolecular complex of fenbendazole with polyvinylpyrrolidone. J Adv Vet Anim Res 2019; 6(1):133–141.

Fenbendazole is an old benzimidazole anthelmintic with a broad spectrum of action that is often used for the treatment of numerous intestinal helminthosis of animals. It is effective at doses of 7.5–10 mg/kg against nematodosis, at the dose of 15 mg/kg against protostrongylide lungworms, at the dose of 100 mg/kg against *Fasciola* spp. infection and *Dicrocoelium dendriticum* infection of sheep [4,5]. The mechanism of action is associated with degradation of microtubules of helminths and violation of glucose uptake in nematodes. Reduction of glucose uptake causes a decrease in energy reserves resulting in the death of the parasite. Fenbendazole is considered safe and non-toxic anthelmintic but the cases of diarrhea and vomiting have been registered [4]. It is known that fenbendazole belongs to the IV class of drugs with low permeability and solubility according to the biopharmaceutical classification of FDA, i.e., has poor bioavailability [6,7]. Consequently, this anthelmintic needs technologies to increase its water solubility.

Modifications and control of solubilizing characteristics of drugs are the most often used parameters in creation and development of modern drug delivery systems [13–15]. Delivery systems of drug molecules with various carriers are in demand. One of the most commonly applied carriers for this purpose is cyclodextrins, which form supramolecular systems [9,10]. Other substances—polysaccharides, liposomes, micelles, and nanoscale inorganic particles can be used as carriers too [11]. The increased pharmacological effect of them is achieved by increasing solubility [12] and membrane permeability [11], as well as improved delivery of drug molecules to the active sites of appropriate receptors. Besides various physicochemical methods are used to increase the solubility of drugs: reduction of particle size, modification of the crystal structure, preparation of solid dispersions of drug substances with fillers, etc. [13–15].

The search of ways to improve the efficacy of fenbendazole and expand its spectrum of action by using of mechanochemical approaches, “guest-host” complexation methods [16] and nanotechnology techniques to improve the solubility, permeability, and eventually the bioavailability of fenbendazole was of great interest. In previous years, we successfully studied the activity of the supramolecular complex of fenbendazole with arabinogalactan in nematodosis of sheep and cattle [17,18]. The purpose of our research was to evaluate the anthelmintic efficacy of the SMCF obtained by the technology of mechanochemical modification of substance with polyvinylpyrrolidone polymer in activators of impact-abrading type—ball mills.

Materials and Methods

Ethical statement

The blinded, randomized, and placebo-controlled study was performed according to the Guidance for the

experimental study of new pharmacological substances [19], the rules adopted by European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes [21] and the rules of good clinical practice of the Russian Federation [20]. Council on Ethics at the Ministry of Health of Russia approved the trials. Clinicaltrials.gov registration protocol №1-110; 14.01.2017.

Preparation of supramolecular complex of fenbendazole

Sample preparation is quite simple and solid phase was conducted in one stage of mechanochemical treatment of fenbendazole and polymer soluble in water—PVP (1:10). The type of mill is a Ball Drum Mill LE-101 (Hungary). The processing mode: 4 h at 60 rpm, grinding media—metal balls (diameter of 23 mm, 1,700 gm), and weight of the material—110 gm. The physical mixture of fenbendazole and PVP 1/10 (without mechanochemical processing) was also prepared.

Characterization

Physicochemical properties and solid phase changes of obtained complexes were evaluated by methods of granulometric analysis, infrared spectral studies; thermal analysis; scanning electron microscopy; the analysis of solubility; and X-ray analysis. These methods were described previously in detail [8,22–24].

Study animals

H. nana and *T. spiralis* infection of mice

The study on the efficacy of the SMCF against *H. nana* infection was conducted on 70 inbred white mice BALB/c (genotype—b, c, H-2d) of both sexes (16–18 gm). Experimental infection with *T. spiralis* was studied on 70 white mice of 1.5–2 months old from Branch Stolbovaya FSBES of NCBMT FMBA of Russia. Mice were quarantined for 7 days before the experiment and received the rat diet (LLC Laboratorkorm, Moscow, Russia) according to the daily feed rate of the Russian Federation [25]. Water was available *ad libitum* throughout the experiment. The mice were in temperature- and humidity-controlled vivarium (22°C, 60%–70%) with natural and artificial light. Random selection was used for group allocation of mice with the same body weight (arithmetic mean 17.36 gm).

Experimental infection of mice and feces examination

The method of sedimentation of B.A. Astafyev et al. [26] was applied to determine eggs of *H. nana*. About 200 infective eggs of *H. nana* were administered orally with a disposable 1 ml syringe with a long needle (25 mm). For this, cestodes of *H. nana* collected from the previous infection were destroyed in a small volume of tap water by repeatedly sucking into a syringe with a needle-cannula. On the

5th day after administration, the feces were examined daily for eggs of *H. nana*.

Infective larvae of *T. spiralis* were isolated by digestion of muscle tissue of rats. Samples were exposed to a digestive fluid (20 ml concentrated HCl, 1,000 ml saline, and 20 gm pepsin at 37°C) for 12 h under continuous mixing with a mechanical stirrer. Then after double sedimentation (1,000 rpm for 2 min), a stable suspension of the sediment larvae was obtained by adding 1.5% gelatin in saline. A chemocytometer was used to count the number of isolated larvae. Mice fasted for 12 h before larvae intra-gastric administration with a tuberculin syringe. The dose for each mouse contained approximately 200 larvae [27].

Helminthosis of sheep

The anthelmintic activity of SMCF was studied in sheep farms in the Moscow and Samara regions (Experimental base Kurilovo, LTD Agro resource), where high levels of helminth infection have been registered.

Experiments were conducted during the period of maximum infection of animals in 2016–2017. On the whole, 319 young sheep of different breeds weighing from 15 to 35 kg were used in the experiments, including 47 naturally infected with *Dictyocaulus filaria*, 59 with *Nematodirus* spp., 53 with other types of gastrointestinal strongylates, 47 with *Strongyloides* spp., 53 with *Trichuris ovis*, and 60 with *Moniezia* spp.

Sheep were kept in stalls (zero grazing) and fed according to the norms of feeding livestock [28]. Water was available *ad libitum* during the experiment.

Random distribution of sheep into the experimental groups with the same number of eggs/larvae per gram of feces was provided by the quantitative fecal egg count (McMaster technique [29] and Baermann techniques [30]) before the study. The geometric mean of eggs/larvae of helminths per gram of feces was counted [31].

Materials

Fenbendazole (Methyl 5-(phenylthio)-2-benzimidazole carbamate), 99.0%, average $M_w \sim 299.35$ was purchased from Changzhou Yabang Pharmaceuticals Co. Ltd (Jiangsu, China). Polyvinylpyrrolidone (PVP) powder, average $M_w \sim 55.000$ ((C₆H₉NO)_n) was purchased from Sigma-Aldrich (St. Louis). All other chemicals used in this study were of reagent grade and were used as received without any further purification.

Experimental groups

Experimental infection with *H. nana*

On the 13th day after infection, six experimental groups and one control group of 10 animals were selected randomly by results of feces examination by McMaster method

[29]. The first, second, and third experimental groups of mice were administered the SMCF via the stomach tube in a dosage rate of 3.0, 2.0, and 1.0 mg/kg of active substance—fenbendazole on 1% starch gel, respectively. The 4th and 5th experimental groups received the substance of fenbendazole at doses of 5 and 1.0 mg/kg of b/w. Physical mixture of fenbendazole/PVP 1/10 was given to the animals of the 6th group at the dose 1 mg/kg of the active substance. The control group of animals received 1% starch gel. On the 4th day after administration, animals were sacrificed by cervical dislocation.

Experimental infection with *T. spiralis*

On the 3rd day after infection, six experimental groups and one control group of 10 animals were randomly selected. The first, second, and third experimental groups of mice were administered the SMCF intragastrically in a dosage rate of 3.0, 2.0, and 1.0 mg/kg of active substance—fenbendazole on the starch gel. White mice of the 4th and 5th groups were given a substance of fenbendazole (without mechanochemical treatment) at doses of 5 and 1 mg/kg on 1% starch gel, respectively. Physical mixture of fenbendazole/PVP 1/10 was given to the animals of the 6th group at the dose of 1 mg/kg. The 1% starch gel was given in appropriate volume to the control group of animals. On the 3rd day after administration of drugs, mice were sacrificed by cervical dislocation.

Sheep were divided into six equal groups of 7–10 animals each by the results of feces examination by McMaster technique [29] and Baermann techniques [30] at each helminthosis. The animals of the first, second, and third groups received the SMCF in the form of 10% powder once at doses of 1.0, 2.0, and 3.0 mg/kg of b/w, respectively. The 4th and 5th groups of sheep were administered a substance of fenbendazole at doses of 1.0 and 5.0 mg/kg, respectively. The control group received no preparation.

Procedures

Examination of mice intestines at helminthological necropsy was conducted on the 4th day after drug administration to evaluate the efficacy of SMCF against *H. nana* and *T. spiralis* infection of mice. Isolated cestodes were counted. Method modified by Denham was used to recover adult worms of *T. spiralis* from the intestines of mice [32].

The efficacy of SMCF in helminthosis of sheep was evaluated by the results of fecal examination methods (McMaster egg count and Baermann method in *D. filaria* infection) before and 15–18 days post-treatment count [29,30]. Identification of parasites was based on the distinct morphology of their fecal forms. The efficacy rate was determined by the percent reduction in geometric mean fecal egg counts between the pre-treatment fecal sample and the post-treatment fecal sample [31].

Statistical analysis

Statistical estimation was conducted on a geometric mean of helminths with parametric *t*-test (significance $p \leq 0.05$). SAS/Stat Software Windows №9.4 was used.

Results and Discussion

As known, fenbendazole is a broad spectrum benzimidazole anthelmintic, very poorly soluble in water. Fenbendazole is metabolized after absorption to oxfendazole and sulfone. The amount of unchanged fenbendazole excreted in the feces is 44%–50% and in the urine is less than 1% in sheep and cattle. Metabolized oxfendazole partially released back to the rumen, where the bacterial flora converted it to fenbendazole. This improves the bioavailability of fenbendazole in ruminants. The excretion half-life of fenbendazole in monogastric and birds is shorter that may require additional doses or more regular appointments of a drug to achieve improved anthelmintic activity [33,34]. In our study, we aimed to increase the solubility of this drug and as a consequence to improve the efficacy of fenbendazole and expand its spectrum of action. Polyvinylpyrrolidone (PVP) is the polymer of vinylpyrrolidone with a high biocompatibility and it is long time used as a delivery system of poorly soluble drugs [35,36] and to control the rate of drug release for improving its pharmacokinetics [37]. The method of mechanochemical modification of fenbendazole was used in the experiment by obtaining solid dispersions with PVP which form supramolecular complexes at dissolving in water or physiologically active media. The data of physicochemical methods demonstrated the formation of supramolecular complexes. X-ray analysis revealed significant differences between the composition of fenbendazole and PVP. The intensity of the crystalline phase of SMCF decreased and its reflexes disappeared in comparison with fenbendazole (Fig. 1). The results indicated a crystallinity loss due to the mechanochemical treatment of the substance and presence of the amorphous state with supramolecular complexes. The reason for this is the increase of the plasticity of PVP that facilitates the mixing of the components on the molecular level. So, Niemczyk et al. [38] showed that the new forms of PVP converted the drug-ibuprofen to be “X-ray amorphous” that expands dissolution a lot. IR-spectroscopy was not informative in our study because of the overlapping spectra of fenbendazole substance and SMCF. Thermal analysis also revealed no information in consequence of significant differences in melting points of compositions. The micrographs demonstrated that PVP consisted of spherical particles with an average size of 10–100 μm (Fig. 2). Fenbendazole consisted of particles with an average size of 5–30 μm (Fig. 3). After mechanochemical processing, the initial fenbendazole particles and spherical particles of PVP were destroyed and

a polydisperse powder was formed, consisting of particles and their aggregates with an average size of 5–20 μm (Fig. 4). Thus, the substance and polymer were crushed to aggregates of irregular shape which promotes the interaction of anthelmintic and PVP.

The solubility of SMCF was increased by 2.8 times after mechanochemical processing. Granulometric analysis of aqueous suspensions of fenbendazole and its composition with PVP showed a large amount of fine fractions in the mass (volume) with a particle size of <10, <5, <1 μm . Thus, the joint processing of fenbendazole with PVP led to a decrease in the size of undissolved particles (Figs. 5 and 6). Probably, this is along with an increase in solubility, this factor can also serve to increase the anthelmintic activity of the obtained drugs. The formation of the

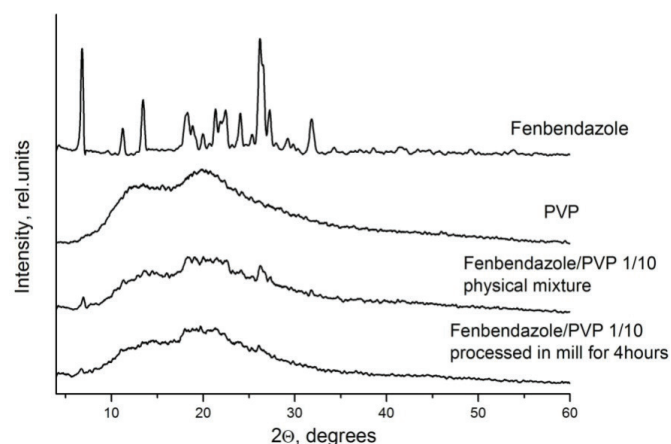


Figure 1. Radiographs of XRA of fenbendazole and PVP, their physical mixture, and mechanochemically obtained composition.

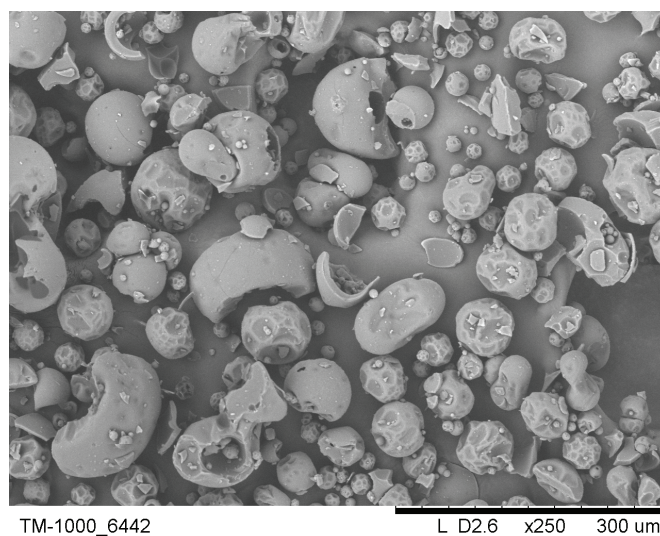


Figure 2. Electron micrographs of the initial substance of PVP.

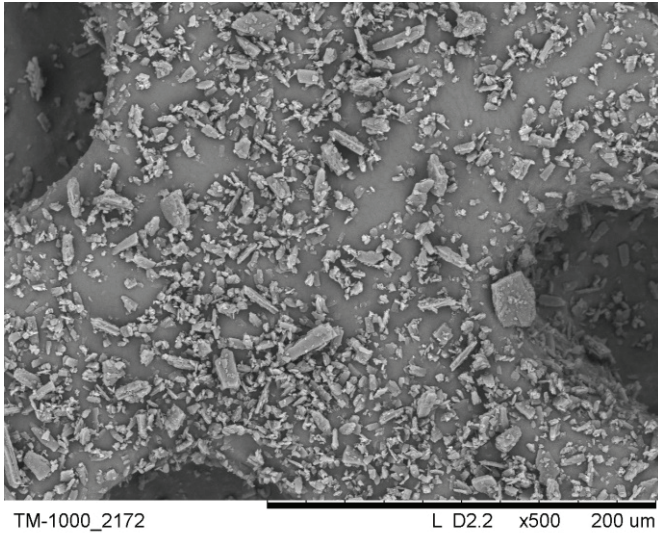


Figure 3. Electron micrographs of the initial substance of fenbendazole.

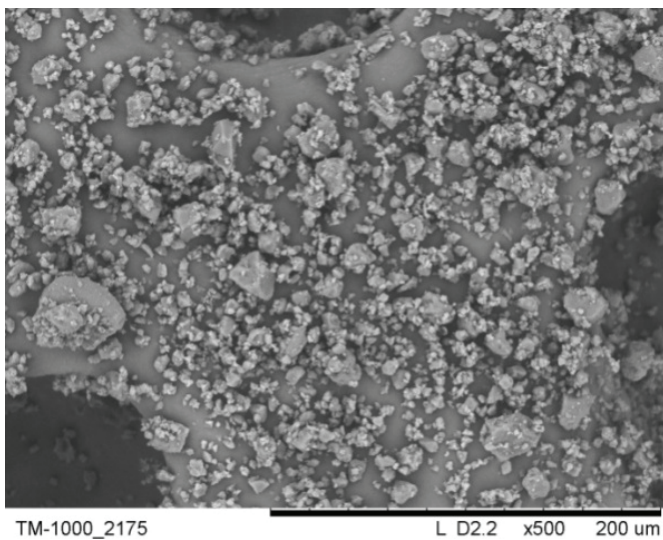


Figure 4. Electron micrographs of the composition of fenbendazole and PVP obtained by mechanochemical technology (1/10 SC 8 h).

supramolecular complex of fenbendazole with PVP occurs according to the “guest-host” scheme described earlier [39,40]. So, hydrophobic interactions of “guest-host” type could facilitate the creation of supramolecular complexes where “guest” is the molecules of fenbendazole and the polymer is a “host” acting as a medication carrier. The data of HPLC showed no chemical interactions or destruction between fenbendazole and PVP after mechanochemical treatment (on the drug concentration and molecular mass characteristics).

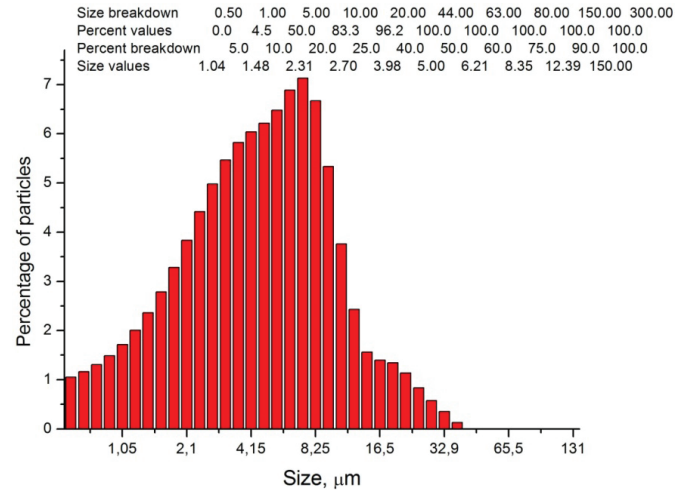


Figure 5. Granulometric analysis of fenbendazole.

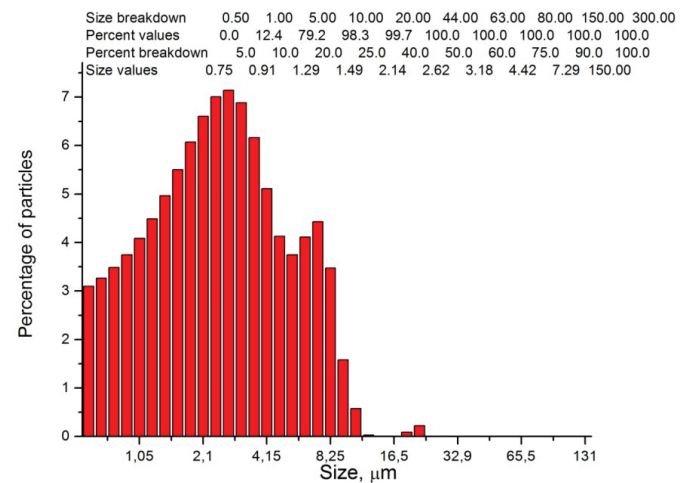


Figure 6. Granulometric analysis of the composition of fenbendazole and PVP obtained by mechanochemical technology (1/10).

Physicochemical methods showed increasing the solubility of SMCF, reduction on the size of particles of initial fenbendazole, disordering of its crystallinity, and generation of the amorphous state with supramolecular complexes. Generally, fenbendazole is evenly located on the surface and in the pores of PVP that changes the medicinal properties and delivers the targeted drug by releasing fenbendazole and its transport through cells membranes.

Fenbendazole is still widely used throughout the world [41–44] in the recommended doses. In our studies, the supramolecular complex of fenbendazole has shown higher anthelmintic efficacy in a reduced dose of 2 mg/kg compared with the original drug on experimental models

Table 1. *Hymenolepis nana* geometric counts, efficacy, and statistical significance for the treatment group ($n = 10$).

Group of animals	Supramolecular complex and its composition	Dose, mg of active substance/kg of BW	Geometric mean of worms	Efficacy ^a (%)	p -Value
Control	Placebo	–	21.032	NA	NA
Treatment	SMCF	3.0	0	100	<0.0001*
Treatment	SMCF	2.0	0.524	97.51	<0.001*
Treatment	SMCF	1.0	1.275	93.94	<0.001*
Treatment	Fenbendazole substance	5.0	1.464	93.04	<0.001*
Treatment	Fenbendazole substance	1.0	19.356	7.97	>0.05
Treatment	Physic. mixture fenbendazole/PVP 1/10	1.0	15.562	26.00	<0.05*

^aPercent efficacy based on geometric means.

*Statistically significant at $p \leq 0.05$ when geometric means were compared to placebo.

Table 2. *Trichinella spiralis* geometric counts, efficacy, and statistical significance for the treatment group ($n = 10$).

Group of animals	Supramolecular complex and its composition	Dose, mg of active substance/kg of BW	Geometric mean of worms	Efficacy ^a (%)	p -Value
Control	Placebo	–	97.863	NA	NA
Treatment	SMCF	3.0	0.054	99.94	<0.0001*
Treatment	SMCF	2.0	0.125	99.87	<0.0001*
Treatment	SMCF	1.0	1.416	98.56	<0.001*
Treatment	Fenbendazole substance	5.0	1.672	98.29	<0.001*
Treatment	Fenbendazole substance	1.0	89.717	8.33	>0.05
Treatment	Physic. mixture fenbendazole/PVP 1/10	1.0	74.362	24.01	<0.05*

^aPercent efficacy based on geometric means.

*Statistically significant at $p \leq 0.05$ when geometric means were compared to placebo.

of helminthosis caused by *T. spiralis* and *H. nana* and on naturally infected sheep by some helminthosis.

Infection of the control group of animals was confirmed by detection of 21.032 adults of *H. nana* in the small intestines of mice at necropsy. All experimental groups had a statistically fewer number of *H. nana* compared with the control group ($p < 0.0001$). So, 0.524 and 1.275 *H. nana* were revealed in the small intestine of mice after treatment of SMCF at doses of 2.0 and 1.0 mg/kg, respectively. This study showed 97.51% efficacy of SMCF at the dose of 2 mg/kg; 93.94% at the dose of 1 mg/kg. Fenbendazole (substance) revealed 93.04% and 7.97% efficacy against *H. nana* at doses of 5.0 and 1.0 mg/kg of b/w, respectively. Physical mixture of fenbendazole/PVP 1/10 demonstrated 26.0% efficacy at the dose 1 mg/kg of active substance (Table 1).

Studies have shown high efficacy of SMCF against *T. spiralis* in white mice (Table 2). The supramolecular complex demonstrated 99.94% anthelmintic activity at the dose of 3.0 mg/kg. On average, 0.125 and 1.416 worms of *T. spiralis* were in the small intestine of mice after SMCF treatment at doses of 2.0 and 1.0 mg/kg with 99.87% and 98.56% of efficacy, respectively. Unmodified fenbendazole showed 98.29% and 8.33% against *T. spiralis* at

doses of 5.0 and 1.0 mg/kg of b/w. Physical mixture of fenbendazole/PVP 1/10 revealed 24.01% efficacy at the dose of 1 mg/kg. An adequate infection was confirmed by detection of 97.863 adults of *T. spiralis* in the small intestines of mice.

The obtained results are shown in Table 3 and indicate different degrees of the efficacy of SMCF at different doses against helminthosis of sheep.

The SMCF demonstrated 100%, 98.22%, and 95.54% efficacy against *Nematodirus* spp. infection at doses of 3.0, 2.0, and 1.0 mg/kg, respectively, according to coproscopical examinations of feces by McMaster technique. After drug administration, 8 out of 10 sheep were also free from infection at the dose of 2.0 mg/kg and the number of eggs decreased to 98.22% in feces. The SMCF showed 95.54% efficacy at the dose of 1.0 mg/kg and 6 out of 10 animals were free from infection. The basic drug—the substance of fenbendazole showed 95.66% efficacy at the dose of 5.0 mg/kg of b/w and 16.49%—at the dose of 1.0 mg/kg.

The SMCF showed 100%, 97.92% and 95.35% efficacy against other gastrointestinal strongylates at doses of 3.0, 2.0, and 1.0 mg/kg of b/w, respectively (Table 3). The substance of fenbendazole revealed 95.73% and 20.54%

Table 3. The spectrum of SMCF activity in helminthosis of sheep.

The drug	Dose, mg of active substance/kg of b/w	Number of sheep in group	Free from infection, head	The number of larvae/eggs in a gram of feces		Decrease in the number of larvae/eggs in feces, %*
				Before treatment	After treatment	
<i>Nematodirus</i> spp.						
SMCF	3.0	10	10	163.4 ± 6.1	0	100
SMCF	2.0	10	8	168.0 ± 5.7	3.0	98.22
SMCF	1.0	10	6	170.5 ± 6.1	7.5 ± 1.7	95.54
Fenbendazole	1.0	10	0	161.8 ± 5.2	140.3 ± 4.3	16.49
Fenbendazole	5.0	10	6	166.5 ± 5.8	7.3 ± 1.8	95.66
Control	-	9	0	161.5 ± 5.7	168.0 ± 6.7	-
Gastrointestinal strongylates						
SMCF	3.0	9	9	186.2 ± 6.4	0	100
SMCF	2.0	9	7	180.6 ± 6.3	3.9 ± 1.0	97.92
SMCF	1.0	9	5	188.1 ± 6.0	8.7 ± 1.6	95.35
Fenbendazole	1.0	9	0	179.6 ± 5.9	148.6 ± 5.0	20.54
Fenbendazole	5.0	9	6	184.8 ± 5.2	8.0 ± 1.5	95.73
Control	-	8	0	182.4 ± 6.4	187.0 ± 6.4	-
<i>D. filaria</i>						
SMCF	3.0	8	8	114.2 ± 4.8	0	100
SMCF	2.0	8	7	113.6 ± 5.3	3.0	97.51
SMCF	1.0	8	5	112.0 ± 4.6	25.6 ± 1.7	87.03
Fenbendazole	1.0	8	0	116.0 ± 4.9	106.2 ± 4.4	11.65
Fenbendazole	5.0	8	6	115.4 ± 4.8	4.5 ± 0.4	96.26
Control	-	7	0	114.2 ± 4.7	120.2 ± 4.8	-
<i>Strongyloides</i> spp.						
SMCF	3.0	8	8	109.6 ± 5.2	0	100
SMCF	2.0	8	5	108.4 ± 5.0	29.1 ± 2.4	74,70
SMCF	1.0	8	1	107.2 ± 4.7	76.6 ± 3.2	33,40
Fenbendazole	1.0	8	0	110.7 ± 3.9	95.0 ± 3.5	17,40
Fenbendazole	5.0	8	5	111.2 ± 3.3	5.2 ± 1.0	95,48
Control	-	7	0	110.8 ± 3.7	115.0 ± 3.5	-
<i>Moniezia</i> spp.						
SMCF	3.0	10	10	169.2 ± 4.2	0	100
SMCF	2.0	10	7	168.0 ± 4.1	4.6 ± 0.6	97.37
SMCF	1.0	10	6	170.1 ± 4.0	8.5 ± 0.6	95.43
Fenbendazole	1.0	10	0	171.2 ± 4.9	148.4 ± 4.7	15.20
Fenbendazole	5.0	10	5	169.6 ± 4.5	16.5 ± 1.6	90.58
Control	-	10	0	170.3 ± 4.7	175.0 ± 4.3	-
<i>Trichuris</i> ovis						
SMCF	3.0	9	5	107.2 ± 2.3	13.3 ± 0.7	88.34
SMCF	2.0	9	3	110.6 ± 2.2	46.7 ± 1.7	59.04
SMCF	1.0	9	1	109.8 ± 2.0	76.3 ± 2.2	33.07
Fenbendazole	1.0	9	0	108.3 ± 2.7	100.2 ± 2.3	12.11
Fenbendazole	5.0	9	3	109.5 ± 2.8	39.0 ± 1.4	65.79
Control	-	8	0	110.3 ± 2.4	114.0 ± 2.6	-

*Statistically significant at $p \leq 0.05$ when geometric means were compared with placebo (control).

efficacy at doses of 5.0 and 1.0 mg/kg, respectively. On average, 182.4 ± 6.4 and 187.6 ± 6.4 eggs of strongylate were in 1 g of feces at the beginning and end of the experiment ($p > 0.05$). Thus, the efficacy of the supramolecular

complex was at 4.6–5.8 times higher than the efficacy of substance.

The efficacy of tested sample was 100%, 97.51%, and 87.03% against *D. filaria* infection at doses of 3.0, 2.0,

and 1.0 mg/kg of b/w, respectively (see Table 3). The substance of fenbendazole showed 96.26% and 11.65% efficacy at doses of 5.0 and 1.0 mg/kg, respectively. The anthelmintic action of SMCF increased by 7.4 times compared with the substance of fenbendazole at the dose of 1.0 mg/kg.

The SMCF showed 100% efficacy against *Strongyloides* spp. infection of lambs at the dose of 3.0 mg/kg of b/w (Table 3). The doses of 2.0 and 1.0 mg/kg revealed 74.7% and 33.40% efficacy, respectively. The basic drug—the substance of fenbendazole showed 95.48% and 17.40% efficacy at doses of 5.0 and 1.0 mg/kg. The infection of animals in the control group did not change significantly during the experiment ($p > 0.05$).

The present study revealed a low efficacy of SMCF against *T. ovis* infection of sheep at doses of 1.0 and 2.0 mg/kg of b/w (Table 3). The efficacy was 88.34% with increasing the dose of SMCF to 3.0 mg/kg. The basic fenbendazole showed 65.79% and 12.11% efficacy at doses of 5.0 and 1.0 mg/kg, respectively. The efficacy of SMCF was 2.7 times higher the efficacy of the substance of fenbendazole at the dose of 1.0 mg/kg of b/w.

No animal was completely free from helminths at studying the efficacy of SMCF at doses of 2.0 and 1.0 mg/kg against *Moniezia* spp. infection. SMCF was found to be 100% effective at the dose of 3.0 mg/kg. Efficacy of the substance of fenbendazole (90.58% and 15.2%) was observed at doses of 5.0 and 1.0 mg/kg, respectively. There was a significant increase of anthelmintic properties of the tested sample compared to the substance of fenbendazole.

Thus, SMCF has shown a higher anthelmintic efficacy at the dose of 2 mg/kg in comparison with the original drug on experimental models of helminthosis caused by *T. spiralis* and *H. nana* and on naturally infected sheep by some helminthosis. These results indicate that the supramolecular complex of fenbendazole with PVP is a promising anthelmintic agent with enhanced efficacy.

Conclusion

The work presented herein has demonstrated a high anthelmintic activity of the novel supramolecular complex of fenbendazole in *H. nana* and *T. spiralis* infection of mice and some helminthosis of sheep in a reduced dose of 2.0 mg/kg. While the substance of fenbendazole showed efficacy at the dose of 5.0 mg/kg. Anthelmintic properties of fenbendazole were enhanced and expanded by increasing of its solubility by mechanochemical modification of the substance with a polymer. Thus, the mechanochemically obtained supramolecular complex of fenbendazole with PVP polymer can serve as a basis

for creating innovative drugs for the treatment of helminthosis in reduced doses.

Acknowledgments

The authors wish to express their gratitude to the technical support of specialists of sheep farms in the Moscow and Samara regions (Experimental base Kurilovo, LTD Agro resource).

This research was conducted within the State Assignment to ISSCM SB RAS (project 0301-2019-0008).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' contribution

Salavat S. Khalikov, Alexander V. Dushkin, and Elizaveta S. Meteleva developed and prepared samples of SMCF, evaluated their physicochemical properties. Irina M. Odoevskaya has led experiments on laboratory model of hymenolepiosis and trichinellosis of white mice. Konstantin M. Sadov, Nataliya V. Danilevskaya, and Anastasiya I. Varlamova studied the efficacy of SMCF on helminthosis of sheep. Ivan A. Arkhipov designed the study, interpreted the data, and drafted the manuscript. Alexander V. Dushkin and Salavat S. Khalikov took part in preparing and critical checking of this manuscript.

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