

Original Article



Laboratory Study of Levamisole on Different Plants in Medicinal Care

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ABSTRACT

Levamisole can be used in a large number of domestic animals such as sheep, goats, cattle, horses, pigs, poultry, dogs and cats as well as wild animals as a drug completely effective in the neonatal and pubertal stages of most gastrointestinal and respiratory nematodes. Animals such as *Homuncus*, *Strategica trichostrongilus*, and *Coperia* are known. Levamisole also has a significant effect on *dictiococcus pneumoniae*, and is the only anthelmintic in sheep for use against lungworms. 15 mg per kg orally and 15 days later an injectable dose will kill the worm and its eggs. Vamisol has little effect on small or large estrogens. In dogs, a dose of 15 mg per kg reduces toxocaracinos, and ascariasis by 91%, and also repels hookworms. In cats, at a dose of 8 mg per kg, it is effective against ascariasis and hookworm. In poultry, it causes a 95% reduction in *Scardidia galli*, and *Hethrax gallinarum* and capillary, if consumed in the amount of 36-49 mg per kg. In addition to its extensive antiparasitic properties, levamisole, with its known immunogenic properties, increases the resistance of the animal body, and thus helps in the treatment of certain diseases in humans and animals. Levamisole is effective in boosting the immune system by increasing the number and function of T lymphocytes and macrophages and can stimulate antibody production, increase macrophage xenophagy, inhibit tumor growth, and enhance inhibitory cell activity.

Introduction

Pharmacokinetics

Levamisole is rapidly absorbed, whether taken orally or by injection [1]. But injecting it causes a higher blood concentration. The maximum blood concentration is one hour after

injection and its plasma half-life is up to 4 hours. Under alkaline conditions, the drug is hydrolyzed to an insoluble metabolite. Levamisole is rapidly excreted from the body, with 46% excreted in the urine after 12 hours and 32% in the feces within 24 hours [2]. It is primarily a cholinergic drug and by acting on the nervous system of the worm and inhibiting the

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enzyme succinate dehydrogenase, it quickly paralyzes parasites and thus facilitates their separation from the gastrointestinal mucosa and respiration and rapid removal of parasites from the animal [3]. In general, side effects are very rare and include: abnormal movement of the head, licking of the lips, increased salivation, muscle tremors, irritability of the nerves, and sometimes intermittent urination and defecation. The most important side effect of drug use in sheep and goats is death due to drug poisoning [4].

Drug Poisoning

Its toxic unit is 2-3 times its therapeutic unit, so great care must be taken in calculating the correct treatment unit. Of course, its toxic spectrum is less than that of benzimidazoles. It causes mortality in sheep at 90 mg / kg, cattle are more tolerant than sheep, horses are more sensitive than other animals, and injections of 20 mg / kg may cause death. Be. Symptoms of drug poisoning include foaming and saliva from the mouth, bradycardia, muscle tremors, or death due to inability to breathe. Perhaps drugs with nicotine compounds, cholinesterase inhibitors such as organophosphates, and neostigmine may potentiate the effects of levamisole. Concomitant use of the drug with chloramphenicol has caused death in some cases. Livestock can be slaughtered 3 days after stopping the drug. Milk can be milked one day after stopping the drug. Injectable drugs are contraindicated in animals close to slaughter because they cause a tissue reaction. If taken orally, it is 7.5 mg per kg in sheep and goats, and 15 mg per kg in cattle. 5 mg per kg is used orally for dogs and 25-50 mg / kg is used orally for poultry [5].

Method of Work

The plants we used, which were two species of Artemisia, were collected from the foothills. Drying the plant to prevent mold, enzymes and bacteria and chemical changes that result in keeping its chemical compounds stable and easy to crush for extraction. The material is composed of it. To dry, the plants were placed in the shade for several days at normal temperature to dry completely [6]. After drying,

the plants were passed through a sieve and the parts used were separated and passed through a sieve again so that the excess parts, skewers and debris were well separated and the extraction was not disturbed. After separation, the parts used by the electric grinder were finely chopped. Grinding is done to increase the contact surface of the particles with the related solvents [7].

Preparation of Plant Extracts

There are several different methods for extracting plant extracts, and here the soaking method was chosen to prepare the extract from this plant. Soaking is an old method that is done with water or various solvents. In our experiments, three types of solvents including: water, ethanol and methanol were used to prepare three types of aqueous extracts, ethanol and methanol. To prepare all three types of extracts from both species of Artemisia plant, 50 grams of the plant in a volume of about 200 cc The solvent was soaked for 48 hours. The soaked solution was then filtered through a Buchner funnel connected to a vacuum pump. The filtered solution was concentrated in a vacuum by a distillation apparatus (about 1-2 hours). The concentrated solution was placed in an oven at 40-50 ° C to dry, which took about 3 days. The extract was stored at 4 ° C until use [8-10].

Preparation of Plant Extracts with Different Concentrations

Preparation of Aqueous Extracts

To prepare 3 different concentrations of aqueous extract, 0.5, 1.5 and 1.5 g of the extract were dissolved in 20 cc of a suitable solvent (PBS was used here), respectively, and concentrations of 25, 50 and 70 mg were Per milliliter of solvent was obtained [11]. To obtain 3 concentrations of methanolic extract, we first dissolved the values of 0.5, 1.5 and 1.5 g of the extract in 1 cc of DMSO and added 19 cc of PBS. Thus, we obtained concentrations of 25, 50 and 75 mg per ml of solvent [12]. Doses of 0.5, 1.5 and 1.5 g of extracts were first dissolved in 1 cc of DMSO solvent and 19 cc of PBS were added. Concentrations of 25, 50 and 75 mg / ml of solvent were obtained. Levamisole was used as a positive control for each series of experiments.

In all experiments, levamisole was prepared in 3 concentrations of 5.50 and 500 mg / ml. Here, too, 20 cc of PBS was used as solvent. Different concentrations were prepared by tubular dilution method [13]. To separate the parasites from the surface of the abomasum, first a very gentle stream and even PBS drip was used. This loosened the parasites' attachments to the surface of the abomasum, while removing the contents of the abomasum that were attached to the parasites and the surface of the abomasum. Then PBS was used with higher flow and the parasites were separated from the surface of the abomasum along with PBS and entered the plate. Among these isolated parasites, healthier, better-moving parasites were selected and placed in fresh PBS. Transfer to fresh PBS removes tissue appendages and the contents of the abomasum attached to the parasites. The parasites were then selected after confirming their sex and species and placed on plates containing extracts and positive and negative evidence [14]. The following plates were prepared for each of the extracts and then 10 healthy parasites were added to each plate [15].

Plate 1- Aqueous, methanolic or ethanolic extract with a concentration of 25 mg / ml.

Plate 2 - Aqueous, methanolic or ethanolic extract with a concentration of 50 mg / ml.

Plate 3- Aqueous, methanolic or ethanolic extract with a concentration of 75 mg / ml.

Plate 4 - Levamisole control with a concentration of 5 micrograms per milliliter.

Plate 5 - Levamisole control with a concentration of 50 micrograms per milliliter.

Levamisole control plate with a concentration of 500 micrograms per milliliter.

Plate 7- PBS control containing 20 cc of PBS solution.

In the case of aqueous extracts, these were 7 plates, but in the case of ethanolic and methanolic extracts, in addition to these 7 plates, we also had 1 other plate, which includes:

Plate 8 - DMSO + PBS control containing 19 cc PBS and 1 cc DMSO.

After preparation, the plates were heated to 25-30 ° C. Evaluation of the movement of parasites to check whether they are alive or dead was performed at one hour intervals for each plate and the results were recorded. The studies were performed for 7-10 hours. The basis of this experiment was the amount of physical movements of the parasites. The parasites that appeared to be dead and immobile were sometimes placed in PBS at a higher temperature (about 35-40 ° C) to make sure that they were alive or dead, and if they did not move, we made sure that they were dead.

Results from Aqueous Extract of Artemisia Siberia

Based on these results, regarding the highest concentration of this extract (1.5 g), all parasites became paralyzed and died after 6 hours. In comparison with the antiphrostatic drug levamisole, the highest concentration of levamisole (500 µg) after 6 hours with It caused the death of all parasites. At a concentration of 1 g of aqueous extract of Artemisia siberia, 100% of the worms died after 9 hours. At the lowest concentration of aqueous extract of Artemisia siberia (0.5 g) after 9 hours, 30% of the parasites were still alive. In the case of PBS control, all parasites were alive throughout the study period [16].

Results of Aqueous Extract of Artemisia Santolia

Concentration of 1.5 g of aqueous extract (Artemisia santolia) after 100 hours caused 100% mortality in parasites. (The mortality that occurred with the highest concentration of levamisole in parasites was the third hour.) The aqueous extract of Artemisia santolina in both medium and low concentrations (1.5 and 0.5 g) after 8 hours caused the death of all parasites. The parasites in PBS were present and healthy throughout the study.

Results of Methanolic Aqueous Extract of Artemisia Siberia

Methanolic extract with the highest concentration (1.5 g) killed all parasites after 3 hours. Regarding the highest concentration of levamisole, all parasites died after 3 hours. The average concentration of methanolic extract of

Artemisia siberia after 4 hours killed all parasites and at low concentrations of the extract (0.5 g) after 7 hours, 90% of the parasites were found dead. In both controls PBS and PBS with DMSO throughout The parasites were healthy during the experiment. Comparison of the effect of methanolic extract of Artemisia siberia with levamisole also shows the effectiveness of this extract on the parasite and its comparability with levamisole [17].

Results from Artemisia Santolina Methanolic Extract

The highest effect was observed in medium concentration of methanolic extract of Artemisia santolia (1 g). However, levamisole caused the death with maximum concentration after 3 hours. In the case of the highest concentration of methanolic extract of Artemisia santolia (1.5 g), all parasites were destroyed after 5 hours. At low concentrations (0.5 g), after 6 hours, 80% of the parasites were eliminated. Comparison of the effect of levamisole and methanolic extract of Artemisia santolia with moderate concentrations shows the effectiveness of the

extract against levamisole against parasites. Unlike the aqueous extract of Artemisia species. Siberia, which seemed to be more effective than the aqueous extract of Santolia, the methanolic extract of Artemisia Santolia had a stronger antiparasitic effect than the methanolic extract of Artemisia Siberia. Of course, a comparison of methanolic extract of Artemisia santolina also shows that methanolic extract is more effective. Artemisia Siberian ethanolic extract in both moderate and high concentrations in the first hour after observation in 100% of parasites caused mortality. And low concentration of ethanolic extract of Artemisia siberia also caused this mortality percentage after 2 hours. However, at the highest concentration, levamisole killed 90% of the parasites after 2 hours. According to the results, the antiparasitic effect of ethanolic extract of Artemisia siberian species is significantly higher than levamisole and if we want to compare the ethanolic extract of Artemisia Siberian with methanolic and aqueous extracts of the same species, its stronger effect than the other two extracts [18].

Table 1 Test results of Artemisia Santonia ethanolic extract

the watch 10	the watch 9	the watch 8	the watch 7	the watch 6	the watch 5	the watch 4	the watch 3	the watch 2	the watch 1	the watch
	50%	80%	100%	100%	100%	100%	100%	100%	100%	Levamisole 5 micrograms per milliliter
	60%	70%	80%	80%	90%	90%	100%	100%	100%	Levamisole 50 micrograms per milliliter
				0	30%	70%	100%	100%	100%	Levamisole 500 micrograms per milliliter
	30%	30%	60%	70%	100%	100%	100%	100%	100%	Aqueous extract 25 micrograms per milliliter
		20%	50%	60%	70%	70%	80%	90%	100%	Aqueous extract 50 micrograms per milliliter
			0	0	40%	60%	80%	90%	100%	Aqueous extract 75 micrograms per milliliter

Table 2 Test results of Artemisia Siberian ethanolic extract

the watch 10	the watch 9	the watch 8	the watch 7	the watch 6	the watch 5	the watch 4	the watch 3	the watch 2	the watch 1	the watch / Extract
90%	90%	100%	100%	100%	100%	100%	100%	100%	100%	Levamisole 5 micrograms per milliliter
50%	60%	80%	80%	80%	90%	90%	100%	100%	100%	Levamisole 50 micrograms per milliliter
				0	50%	70%	100%	100%	100%	Levamisole 500 micrograms per milliliter
20%	40%	70%	70%	100%	100%	100%	100%	100%	100%	Aqueous extract 25 micrograms per milliliter
					100%	100%	100%	100%	100%	Aqueous extract 50 micrograms per milliliter
100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	PBS control
100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	PBS + DMSO control

Table 3 Test results of Artemisia Siberian methanolic extract

the watch 10	the watch 9	the watch 8	the watch 7	the watch 6	the watch 5	the watch 4	the watch 3	the watch 2	the watch 1	the watch / Extract
				100%	100%	100%	100%	100%	100%	Levamisole 5 micrograms per milliliter
			50%	70%	80%	80%	90%	100%	100%	Levamisole 50 micrograms per milliliter
							0	50%	100%	Levamisole 500 micrograms per milliliter
			10%	10%	20%	30%	50%	50%	100%	Methanolic extract 25 mg / ml
						0	10%	20%	100%	Methanolic extract 50 mg / ml
							0	40%	60%	Methanolic extract 75 mg / ml
				100%	100%	100%	100%	100%	100%	PBS control
				100%	100%	100%	100%	100%	100%	PBS + DMSO control

Table 4 Test results of Artemisia Santolina methanolic extract

the watch 10	the watch 9	the watch 8	the watch 7	the watch 6	the watch 5	the watch 4	the watch 3	the watch 2	the watch 1	the watch / Extract
100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	Levamisole 5 micrograms per milliliter
		30%	50%	70%	80%	80%	90%	100%	100%	Levamisole 50 micrograms per milliliter
							0	45%	100%	Levamisole 500 micrograms per milliliter
				20%	20%	50%	80%	100%	100%	Methanolic extract 25 mg /ml
								0	100%	Methanolic extract 50 mg / ml
					0	10%	20%	50%	100%	Methanolic extract 75 mg / ml
100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	PBS control
100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	PBS + DMSO control

Table 5 Test results of Artemisia Siberian ethanolic extract

the watch 10	the watch 9	the watch 8	the watch 7	the watch 6	the watch 5	the watch 4	the watch 3	the watch 2	the watch 1	the watch / Extract
			80%	80%	80%	80%	80%	100%	100%	Levamisole 5 micrograms per milliliter
			50%	50%	50%	50%	50%	50%	100%	Levamisole 50 micrograms per milliliter
								10%	100%	Levamisole 500 micrograms per milliliter
								0	30%	Ethanol extract 25 mg / ml
									0	Ethanol extract 50 mg / ml
									0	Ethanol extract 75 mg / ml
			100%	100%	100%	100%	100%	100%	100%	PBS control
			100%	100%	100%	100%	100%	100%	100%	PBS + DMSO control

Table 6 Test results of Artemisia Santolina ethanolic extract

the watch 10	the watch 9	the watch 8	the watch 7	the watch 6	the watch 5	the watch 4	the watch 3	the watch 2	the watch 1	the watch / Extract
90%	90%	100%	100%	100%	100%	100%	100%	100%	100%	Levamisole 5 micrograms per milliliter
50%	60%	70%	80%	80%	90%	90%	100%	100%	100%	Levamisole 50 micrograms per milliliter
				0	50%	70%	100%	100%	100%	Levamisole 500 micrograms per milliliter
20%	40%	50%	70%	100%	100%	100%	100%	100%	100%	Ethanol extract 25 mg / ml
0	30%	30%	60%	100%	100%	100%	100%	100%	100%	Ethanol extract 50 mg / ml
			0	20%	50%	100%	100%	100%	100%	Ethanol extract 75 mg / ml
100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	PBS control
100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	PBS + DMSO control

Result of Artemisia Santolia Ethanolic extract

Artemisia santolia ethanolic extract in high concentration (1.5 g), after 7 hours, killed all parasites, and levamisole in the highest concentration after 6 hours caused such a mortality rate. Medium concentration of methanolic extract of Artemisia santolia after 10 hours killed all parasites and at low concentration (0.5 g) after 10 hours 80% of the parasites were found dead [19-21]. Comparison of 3 types of aqueous extract, methanolic. Ethanol of Artemisia santolia species shows that methanolic extract had the highest antiparasitic effect, followed by ethanolic and aqueous extracts of this species, respectively. In addition, the antiparasitic effect of Artemisia Siberian ethanolic extract seems to be stronger than the ethanolic extract of Artemisia Santolia according to these results [22-24].

Conclusion

The concentration of both extracts used in the laboratory was 25 mg / ml. Experiments performed in the laboratory showed the effectiveness of both aqueous and methanolic extracts of the plant on the parasite Heminocus

contortus. However, methanolic extract had a stronger effect than aqueous extract. It should be noted that the effect of the extracts was less than that of levamisole, which was used as a control. Thus, in all cases, the parasites were completely dead after 6 hours, but in the case of extracts, after 6 hours, although a significant number of parasites were dead, a small number still survived. In our experiments, methanolic and ethanolic extracts were more effective than the extract. Aqueous showed and perhaps according to these two similar results, it can be said that the active ingredient in the plant, which has an anti-parasitic effect, is better extracted by methanol and ethanol. In Iqbal et al. One milligram per milliliter killed 30 percent of the parasites after 6 hours, and the levamisole used in their experiments killed all the parasites after 6 hours. In our experiments on the aqueous extract of Artemisia siberia, after 6 hours, 30% of the parasites were dead. In our experiment, levamisole caused 100% mortality in parasites after 6 hours. While the highest concentration of levamisole in our experiments was equal to the concentration of levamisole used in the experiments of Iqbal et al. (2004), the novel and the amount of the effect of aqueous extracts of

Artemisia siberia in our experiment and *Artemisia bruifolia* in Iqbal experiment are similar. And for each two times that levamisole killed all the parasites, these two extracts of two species of *Artemisia* killed 30% of the parasites. In the case of the aqueous extract of *Artemisia santulina*, it also killed 30% of the parasites after 6 hours, but with the difference that levamisole killed all parasites after 3 hours, so it has a weaker antiparasitic effect compared to *Artemisia bruifolia*. In the case of the methanolic extract of *Artemisia berivolia*, when levamisole killed all parasites. 80% of the parasites were dead. As for the methanolic extract of *Artemisia siberia*, when levamisole killed all the parasites, the methanolic extract killed 50% of the parasites. Therefore, the methanolic extract of *Artemisia berifolia* seems to be more effective than the methanolic extract of *Artemisia siberia*. The methanolic extract of *Artemisia santolina*, when levamisole killed all parasites, killed only 10% of the parasites, so the methanolic extract of *Artemisia brifolia* was more effective than the methanolic extract of *Artemisia Santolina*. However, it should be noted that these results are related to the concentration of 25 mg per ml of extracts, and at higher concentrations, our extracts showed remarkable results. In the case of the aqueous extract of *Artemisia bruifolia*, the dead parasites showed movement again after being placed in fresh PBS, while in our experiments the dead parasites showed no movement after being placed in fresh PBS at higher temperatures. Did not give. No similar results were observed in comparing the survival of *Parabronma* and *Hemcucus* parasites in PBS medium. In our experiments, *parabronma* parasites had perfectly clear movements in PBS for up to 10 hours after the start of the experiments and survived even in cases where they were kept for 24 hours, but in the experiments of Iqbal *et al.* (2004), some *Homoncus* parasites became paralyzed and died 6 hours after the start of the experiment, indicating that *parabronma* parasites were more resistant *in vitro*. Evidence used also included 86% tween and normal sillin. Earthworms were collected from the soil where the pigs were kept and 2 other parasites were prepared from the pig intestines. The extract and drug used in these experiments were liquid and concentrations of

0.5, 1 and 2% of the drug and extract were used. The laboratory method used by Nakhareh and Gray was the same as the method used by the mother in the laboratory environment. In this experiment, the number of worms used in each dish was 6. The paralysis and death of the worms here were also done by direct observation method and some worms were removed. They were transferred to hot water to ensure their death. According to the results, the extract had a lethal and paralyzing effect on all three types of parasites, and the effect of the anti-parasitic extract was 2-3 times better than that of the anti-parasitic drug. The parasite used was against these parasites. Also, in earthworms, the activity of plant extract was 85% better than the antiparasitic drug piperazine phosphate. In this experiment, the evidence used did not show any antiparasitic effect. Idris *et al.* (1982) The antiparasitic effect of *Artemisia herba powder*. Alba was tested on the same parasites as *Hemocus contortus* and using 6 sheep. The clinical signs of homozygosity, which include dizziness, anorexia, and hard stools, have been studied in this sheep, and of course the symptoms have been consistent with the pathological findings observed. Each sheep has been infected with a round of 1000-800 infected larvae. None of these clinical signs of the disease was observed in 4 of these 6 sheep treated with concentrations of 2, 10 and 30 g of powder. The absence of eggs in the feces, adult worms in the abomasum at necropsy, and significant and significant damage to the tissues of these sheep has been a confirmation of successful treatment. Also, the concentrations of ammonia sodium, potassium, protein and creatinine in the serum have returned to pre-infection levels, which is another confirmation of the antiparasitic effect of *Artemisia herba alba* on the parasite *Hemicoccus contortus*. In the other 2 sheep that remain, although the number of eggs in the feces has decreased and the number of adult worms received in the abyss has decreased, they have not completely disappeared like the other 4 sheep. The effect of some species of this plant against parasites Foreigners have also been examined. Maggie *et al.* (2006) tested the antiparasitic effect of ethanolic extracts of *Artemisia vulgaris* and *Artemisia obsintum* on the skin of a number of pigs. The extract used in

this experiment was prepared using 70% ethanol and the experiments were performed on 6 pigs that were raised from Estoshia farm. The experiments lasted for 5 weeks and the time interval between each treatment was one week. During 2-4 weeks after the start of the experiments, 57-57% of the scabies disappeared. 44% of these deaths were related to the week after the experiment. This result shows the effectiveness of ethanolic extract of *Artemisia vulgaris* and *Artemisia obsintum* against foreign parasites. Due to the anti-parasitic properties of this particular species and on the parabronma scriabin parasite, no scientific experiment has been performed to date and this research is the first experiment in this field.

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