



Received on 11 March, 2017; received in revised form, 10 June, 2017; accepted, 17 September, 2017; published 01 October, 2017

## IN VITRO EFFECT OF SOME IRANIAN MEDICINAL PLANTS ON THE *HISTOMONAS MELEAGRIDIS* PARASITE

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### Keywords:

*Histomonas meleagridis*,  
Medicinal herbs, Metronidazole,  
*Dracocephalum kotschyi*

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**ABSTRACT: Objective:** This study aimed to investigate the *in vitro* effect of the extracts of five Iranian medicinal herbs on *Histomonas meleagridis* protozoan parasite. **Methods:** This study was conducted in the following three phases in Khorramabad, Iran, in 2016: 1) preparing and extracting the 5 medicinal herbs *Dracocephalum kotschyi*, *Satureja khuzestanica*, *Pulicaria gnaphalodes*, *Olea europaea*, and *Echinophora platyloba* DC; 2) culturing the positive cases of fecal samples in the fortified commercial medium RPMI<sub>1640</sub> up to  $1 \times 10^6$  parasite/ml of the parasite; and 3) investigating the effect of 1.25, 2.5, and 5 mg/ml densities of each extract and metronidazole compared with the negative control on hours 2, 4, 6, 24, and 72 with Trypan blue vital stain. **Results:** In this study, the *D. kotschyi* extract which destroyed 80% of the parasites in the first two hours was the most, and the *S. khuzestanica* extract was the second effective extract. However, metronidazole destroyed 60% of the parasites in the first two hours and 100% in the third day and may be excreted before being effective, showing relative resistance. **Conclusion:** The results of the present study demonstrated that herbal medicines may be a natural source for the production of new agent to treat histomoniasis infection.

**INTRODUCTION:** The protozoan parasite *Histomonas meleagridis* (family Monocercomonoidae) which is found in the cecal area of the digestive system of different species of birds lives in the amoeboid form in the tissue and the flagellate form in the cecal lumen<sup>1, 2</sup>. This parasite which is proliferated through binary fission is extremely sensitive and is destroyed outside the body in a matter of hours.

It is transmitted in various ways, the most important of which are the eggs of the cecal nematode of birds (*Heterakis gallinarum*) which keep the parasite alive for two years in proper environmental conditions. The parasite-bearing nematode eggs may be transmitted to other birds directly or by being eaten by earthworms as the vector host<sup>2</sup>.

*H. meleagridis* causes histomoniasis (blackhead) with severe and broad necrosis in the mucous and submucous and parenchymal liver disease, developed in the presence of pathogens including *E. coli* and coccidia<sup>3</sup>, and demonstrating symptoms such as yellow sulfur diarrhea, lack of appetite, decreased growth, thirst, droopy wings, and ruffled feathers 7-12 days after the infection<sup>4</sup>. Turkey are

QUICK RESPONSE CODE	DOI: 10.13040/IJPSR.0975-8232.8(10).4418-22
	Article can be accessed online on: www.ijpsr.com
DOI link: <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.8(10).4418-22">http://dx.doi.org/10.13040/IJPSR.0975-8232.8(10).4418-22</a>	

most sensitive to this disease which leads to death in 80-100% of them and brings about heavy economic losses<sup>5</sup>.

Numerous chemical compounds including arsenic and nitroimidazole compounds have so far been used in the prevention and treatment of histomoniasis. From among the former group, only Histostat (nitarosone) has received FDA approval solely for prevention, and from among the latter, the most effective one, dimetridazole, which has been banned in animals for its carcinogenic, mutagenic, and genotoxic effects<sup>6,7</sup>. As a result, a treatment gap exists for histomoniasis which calls for attention<sup>8</sup>. The use of medicinal herbs which are widely available and inexpensive and have few side-effects may be the answer. So far, studies have been conducted on the effect of oils and ethanol of these herbs on histomoniasis<sup>9-12</sup>. Despite the 16% prevalence of digestive parasites in Lorestan Province, Iran, and the high prevalence of *Blastocystis* in humans and cattle, no study has yet been conducted on the effects of medicinal herbs<sup>12-14</sup>.

The present study aimed at investigating the effect of the extracts of 5 medicinal herbs on the pathogen parasite *H. meleagridis* and comparing them with metronidazole *in vitro*, so that the most effective extract would be identified and its active ingredients determined, condensed, reanalyzed, and used as a compound in the prevention and treatment of histomoniasis.

## MATERIALS AND METHODS:

**Plant Material:** The plant materials of *Dracocephalum kotschyi*, *Satureja khuzestanica*, *Pulicaria gnaphalodes*, *Olea europaea*, and *Echinophora platyloba* DC were collected in April 2016, from the various regions of Lorestan province, Iran. The taxonomic identification of the plant was confirmed by a botanist and voucher specimens were deposited in the Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences (Khorramabad, Iran).

**Preparation of the Extracts:** The dried plant materials were grinded and extracted with water by percolation method 72 hr in room temperature. The extracts were passed through filter paper (Whatman No.3, Sigma, Germany) to remove plant debris.

The extracts were finally concentrated in vacuum at 50°C using a rotary evaporator (Heidolph, Germany) and stored at -20 °C, until use<sup>15-17</sup>.

**Parasite:** *H. meleagridis*-bearing fecal samples were mixed with physiological saline solution. The resulting suspension was filtered by passing through two layers of moist gas, and centrifuged at 5000 rpm/5 min. Then, 1 mL of the suspended sediment was fortified with 4 mL of the commercial culture medium RPMI<sub>1640</sub>, mixed with inactivated horse serum and some rice starch (without antibiotics), and subcultured at 30 °C until 1×10<sup>6</sup> mL of the parasite was achieved.

## Effects of various extracts on *H. Meleagridis*:

Two series of four 2 mL microtubes each (one series for the extract and one for metronidazole) were selected for each extract. 1.5 mL of the RPMI<sub>1640</sub> fortified culture medium and 0.5 mL (1×10<sup>6</sup> parasite/ml) of the parasite-bearing culture (Phase B) were added to each microtube and mixed. Then, 1.25, 2.5, and 5 mg/ml of each herbal extract were added to the first series, and the same amounts of metronidazole were added to the second series, mixed again, incubated at 38 °C, and dyed using Trypan blue vital stain in the hours 2, 4, 6, 24, and 72 h.

**Statistical Analysis:** Data analysis was performed by using SPSS statistical package (version 17.0) (SPSS Inc., Chicago, IL, USA). Differences between test and control groups were analyzed by t-test. *P* <0.05 was considered statistically significant.

**RESULTS AND DISCUSSION:** The effects of various densities of herbal extracts and metronidazole on *H. meleagridis* in different times are demonstrated in **Table 1**. Metronidazole is a nitroimidazole derivative and one of the active compounds affecting parasitic protozoans in humans and animals. It was not much effective in this study since it managed to destroy all the parasites as late as the third day and is certainly excreted from the bird's body before being effective, showing the relative resistance of this parasite to metronidazole. The most effective extract was *D. kotschyi* extract which destroyed almost 80% of the parasites in the first two hours and almost 90-97% in the third day. It was thus more effective than metronidazole in the first 2

hours, although a small percentage of parasites were alive in the third day. The second most effective extract was *S. khuzestanica* extract which destroyed almost 50% of the parasites in the first

two hours and 80 - 85% in the third day. The other three herbal extracts managed to destroy 30 - 40% of parasites and are, therefore, removed from the treatment program.

**TABLE 1: EFFECT OF VARIOUS CONCENTRATIONS OF TESTED EXTRACTS ON VIABILITY OF HISTOMONAS MELEAGRIDIS AT DIFFERENT TIME IN COMPARISON WITH METRONIDAZOLE AS POSITIVE CONTROL**

Time	2h			4h			6h			24h			72h		
Concentration mg/ml	1.25	2.5	5	1.25	2.5	5	1.25	2.5	5	1.25	2.5	5	1.25	2.5	5
Negative control	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Positive control (Metronidazole)	49	60	65	51	60	68	60	69	79	90	93	0	0	0	0
<i>D. kotschi</i>	76	80	80	80	82	85	85	83	88	85	85	90	90	92	97
<i>S. khuzestanica</i>	45	48	48	48	50	55	57	60	65	63	70	80	80	83	85
<i>P. gnophalodes</i>	38	35	40	40	45	49	44	50	58	50	59	65	65	73	80
<i>O. europaea</i>	35	34	39	39	44	45	40	49	50	49	58	59	67	71	71
<i>E. platyloba</i>	30	29	34	30	35	37	37	40	48	42	46	47	51	59	70

Unlike some human worm parasites<sup>18</sup>, the prevalence of *H. meleagridis* has increased from 6.5 to over 30% in the birds of Khorramabad (Lorestan Province, Western Iran) rural areas<sup>19</sup>, due to negligence on the part of veterinarians, lack of knowledge of bird owners, or absence of appropriate medications. The ban on the use of nitroimidazole active compounds (because of their side-effects on animal product consumers) and arsenic compounds in the prevention and treatment of histomoniasis<sup>19, 20</sup> has caused a gap in the treatment of this parasite and its diseases, resulting in the recurrence of histomoniasis<sup>21</sup>.

Moreover, this study showed that this parasite is relatively resistant to metronidazole which is effective in the treatment of many protozoans as a nitroimidazole compound<sup>22</sup>, because it managed to destroy only 60% of them in the first two hours. Although it needs further investigation, other studies have also reported resistance to metronidazole in *Trichomonas*, confirming our result (19). Nevertheless, a previous study has demonstrated that the digestive trichomonads in birds are highly sensitive to metronidazole, contrary to our result<sup>23</sup>.

Currently, a medical compound called Histostat (nitarson) is used in the US in order to prevent histomoniasis and a medicine called Nifursol is used in Europe for the prevention and treatment of this disease, fed to animals as food additives with the ratios of 187.5 and 50-75 ppm, respectively, up to five days before using their products<sup>1, 24</sup>. It is

noteworthy that Histostat which is widely used in the prevention and treatment of histomoniasis is an arsenic compound, not strong enough to be used as a reliable medicine to treat infections<sup>25-27</sup>; moreover, Nifursol is the only nitrofurantoin-containing compound used in the prevention of histomoniasis; other compounds are used in the treatment of animal bacterial infections<sup>28, 29</sup>. As a result, there is no reliable chemical therapy for histomoniasis. Thus, a systematic and fundamental solution to this problem could be the use of easily available natural herbal compounds which can be effective for these useful and beautiful animals which are sick, have diarrhea, and lost their feathers due to this disease. Unfortunately, there are few relevant studies on herbalism.

*H. meleagridis* is a digestive parasite in birds<sup>1</sup>. According to studies, the in vivo and in vitro effects of herbal products and chemical medications on birds' digestive parasites are almost the same<sup>30</sup>. If so, in vitro studies which are easier and less expensive than in vivo studies would be highly useful. Considering the fact that the contents of birds' digestive system move with a constant trend, compounds which work with lower densities and in shorter times are determined as more effective. Other compounds have no treatment applicability and are excreted from the body before being effective.

*D. kotschi* is a fragrant and self-growing plant which is endemic to Iran and is known as Dennaie, Badrandjboie, and Zarrin-giah<sup>31</sup>.

It is used as a traditional medication in the treatment of headaches and digestive and liver disorders, and also as a flavor for tea and yogurt<sup>32</sup>. Recently, its anti-cancer<sup>33</sup>, cytotoxic<sup>34</sup>, and immunoregulatory<sup>35</sup> effects have been identified.

In the present study, its extract was determined as the most effective herbal extract as it managed to destroy 80% of the parasites in the first two hours. It may be 100% effective in higher densities, as it was *in vitro*. However, it may be excreted from the birds' digestive system before it can be effective. The second most effective extract was the *S. khuzestanica* extract which destroyed 45-48% of the parasites in the first 2 hours. The parasite MIC did not reach 100% with this extract either. The other extracts namely *P. gnaphalodes*, *O. europaea*, and *E. platyloba* DC destroyed 30-40% of the parasites and cannot be considered in the treatment of this parasite.

**ACKNOWLEDGMENT:** This study was supported by Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences (Khorramabad, Iran).

**CONFLICT OF INTEREST:** The authors declare that there is no conflict of interests.

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**How to cite this article:**

Badparva E, Badparva S, Mousavi SF and Mahmoudvand H: *In vitro* effect of some Iranian medicinal plants on the *Histomonas meleagridis* parasite. Int J Pharm Sci Res 2017; 8(10): 4418-22.doi: 10.13040/IJPSR.0975-8232.8(10).4418-22.

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