Research Note

IN VITRO EFFECTS OF DECOCTION FROM FIVE SPECIES OF CHINESE PLANTS AGAINST *TRICHOMONAS VAGINALIS*

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Abstract: *In vitro* effects of extracts from the 5 species of Chinese herbal medicine against *Trichomonas vaginalis*, one of the most important protozoal diseases in China, were investigated. It was demonstrated that the extracts from *Artemisia annua*, *Phellodendron amurense* and *Pulsatilla chinensis* were highly effective against the protozoa at 1 to 5 hr after the start of the exposure to the drugs. The extract from *Cnidium monnieri* was much less effective and that from *Sophora flavescens* showed no activity even after the exposure for 5 hr. After this screening for excluding non-effective drugs, the parasites were exposed to all of the drugs except the extract from *S. flavescens*, the negative one in the above test, for 5 days at the drug concentrations lower than those above. The extracts from *A. annua* and *P. chinensis* were highly effective, immobilizing the parasites completely 2 or 3 days after the start of the exposure and afterwards. Contrarily, those from *C. monnieri* and *P. amurense* were not so effective. The results obtained in the present studies were in favour of the prospect that further studies including those on side effects, would be worthy of being carried out for the establishment of the chemotherapy of *T. vaginalis* infection with the crude drugs from medicinal plants in China.

Key words: *Trichomonas vaginalis*, Chinese medicinal plants, therapy

INTRODUCTION

There are a large number of obstinate parasitic diseases still nowadays in Asian and other countries on the earth. Though several excellent drugs have been synthesized for their treatment and control, there still remain matters to be considered. Excellent synthesized drugs are not readily available in local villages because of their high cost and inconveniences in transportation. This fact might be rather a minor one. However, there is an important problem. Some strains (isolates) of parasitic protozoas and helminths have been found to be insensitive to excellent synthesized drugs (Boreham, 1995; Geerts *et al.*, 1997). For instances, according to Boreham (1995), some lines of *Giardia* resistant to quinacrine and albendazole have been isolated.

The situation with these facts is thought to be where the possible development of new drugs would

hopefully come in, based on scientific exploitation of traditional medicinal plants like a global strategy for the control of obstinate parasitic diseases (Maki *et al.*, 1996). This is, at least in part, in accordance with the famous fact that cloroquine-resistant malaria is treated nowadays with quinghaosu, originally a traditional Chinese drug from the plant, *Artemisia annua*.

In this line of thought, the present authors have been testing efficacy of extract from medicinal plants on various kinds of parasites (Maki *et al.*, 1997). Effects of decoction from a number of Chinese plants against *Trichomonas vaginalis* have been one of the topics which interest them.

Much attention has been paid to a large number of Chinese medicinal herbs because of their possible utility for the control of various kinds of parasites (Shen, 1992; Zhao, 1983; Zhao *et al.*, 1991). Traditional medicinal plants, *A. annua*, *Cnidium monnieri*, *Phellodendron*

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Table 1 Efficacy in scores of the extracts from the 5 species of Chinese plants against *Trichomonas vaginalis*

Extract diluted (4 to 64 times)	duration of exposure				
	1 hr	2 hr	3 hr	4 hr	5 hr
<i>A. annua</i>					
4	4	4	4	4	4
8	4	4	4	4	4
16	4	4	4	4	4
32	4	4	4	4	4
64	3	4	3	4	4
<i>C. monnieri</i>					
4	1	1	1	1	2
8	1	1	1	1	1
16	0	0	1	1	1
32	0	0	0	1	0
64	0	0	0	0	0
<i>P. amurense</i>					
4	4	4	4	4	4
8	4	4	4	4	4
16	2	4	4	4	4
32	0	3	3	4	4
64	0	2	2	2	4
<i>P. chinensis</i>					
4	4	4	4	4	4
8	4	4	4	4	4
16	4	4	4	4	4
32	4	4	4	4	4
64	3	4	4	4	4
<i>S. flavescentis</i>					
4	0	1	1	1	0
8	0	0	0	0	0
16	0	0	0	0	0
32	0	0	0	0	0
64	0	0	0	0	0

Number of *T. vaginalis* moving per field of the view (10×10) was recorded with the result of more than 50 parasites in control groups. The efficacy of the extracts tested was expressed in scores in this table. The efficacy score 4, 3, 2, 1 or 0 was given to the groups in which the number of moving parasites was 0, 1-5, 6-10, 11-50 or more than 50, respectively.

amurense, *Pulsatilla chinensis*, and *Sophora flavescentis* were chosen for the present study. These medicinal plants are readily available in China, mostly seen in the fields of North-East area of China.

The clinical utility of the 5 plants for therapy of infectious diseases is described in a number of publications including Japanese books. For example, according to Mitsuhashi (1988) and Namba (1993 a, b), these plants are useful for the treatment of parasitic and bacterial infections as follows. *A. annua* (whole herb) is a plant of established utility for the treatment of malaria as mentioned above. Extract from *C. monnieri* (fruits) is described as a drug having anti-*T. vaginalis*

Table 2 Efficacy of the extract from the 4 species of the plants in the reduction of the number of *T. vaginalis*

Extract diluted (64 to 512 times)	Reduction in %				
	Duration in days after the start of exposure				
	1 day	2 day	3 days	4 days	5 days
<i>A. annua</i>					
64	95	93	<u>100</u>	<u>100</u>	<u>100</u>
128	78	83	72	86	86
256	37	18	56	82	75
512	52	31	5	69	65
<i>C. monnieri</i>					
64	86	84	79	92	88
128	74	72	76	90	85
256	25	41	56	63	59
512	7	37	60	64	57
<i>P. amurense</i>					
64	89	99	83	76	50
128	81	84	70	79	48
256	38	39	58	83	51
512	44	35	69	75	51
<i>P. chinensis</i>					
64	86	<u>100</u>	<u>100</u>	<u>100</u>	<u>100</u>
128	69	98	99	99	84
256	74	65	57	41	23
512	8	39	54	39	33

The mean number of parasites per $10 \mu\text{l}$ of the medium in the control group with standard deviation was originally recorded. However, statistical analysis was not carried out because the purpose of this study was to find such a decoction at a certain concentration that will prohibit the movement of the parasites completely (see underlined parts).

action. One of the components of *P. amurense* (bark), berberine has an activity against bacterial infection. *P. chinensis* (roots) containing saponin is useful for the treatment of *Entamoeba histolytica* infection and malaria. And *S. flavescentis* (roots) is said to have an anthelmintic action.

It is intriguing to find out a possible efficacy of the extract from the plants including *C. monnieri* against *T. vaginalis*. The present communication describes the comparative effects of the plant extracts against *T. vaginalis* cultured in our laboratory.

MATERIALS AND METHODS

Decoction tested were as follows. The commercially available medicinal plants in China, *A. annua*, *C. monnieri*, *P. amurense*, *P. chinensis*, and *S. flavescentis* were washed and dried. Five grams of the materials was added to 100 ml of distilled water. They were each boiled for 30 minutes. The extract was filtered and preserved as the first part of the drug extraction. Fifty

ml of distilled water was added to the residue and this was boiled again. The extract was filtered to obtain the second part of drug extraction. The first and second portion were mixed. The mixed extract was boiled to be concentrated to 10 ml. As a result, 10 ml extraction was prepared using 5 g of the materials. This decoction was diluted at two folds repeatedly up to nine times with sterilized distilled water. For the test in which the parasites were exposed to the extract for 5 hr, it had been diluted at 4, 8, 16, 32 and 64 folds and for the exposure for 5 days at 64, 128, 256 and 512 folds.

T. vaginalis were donated from School of Medicine, Tokai University, Kanagawa Prefecture, Japan. They were maintained in Asami media containing about 1,500 parasites/ml before proliferation at $36.5 \pm 0.5^\circ\text{C}$ throughout the present studies.

A preliminary study was first carried out in order to exclude non-effective drugs among the extracts from the 5 species of the plants. The decoction diluted with sterilized distilled water at a desired concentration (or the water in the controls) and the culture medium containing the parasites were mixed at the ratio of 1:5 throughout the present studies. In the first study, 10 μl of the suspension was taken onto glass slides for their observation at 1, 2, 3, 4 and 5 hr after the mixture had been put into each pore of a plastic plate. Three fields of the light-microscope view (10×10) were checked to count the number of the moving (including "moribund") or immobilized parasites. They were observed sometimes at 10×40 whenever their close examination was necessary. The test was repeated three times for the confirmation of the reproducibility. A special attention was paid as follows to distinguish the moving parasites from the immobilized ones during the preliminary study for 5 hr and the subsequent one for 5 days. The normal parasites like those in control groups were seen rotating actively or going back and forth with the movement of the anterior flagella. The cytoplasm of the normal parasites was clear with transparent granules distributed in the inside. The moribund parasites were attached with more and more materials with the passage of time. The movement of the anterior flagella and undulating membrane became slower and ceased eventually. The cytoplasm of the immobilized parasites contained larger and obscure granules of poor transparency. Their distribution in the cytoplasm was not clear.

The subsequent study was carried out in an attempt to select drugs that immobilize the parasites completely at a certain concentration. In this study, the test tubes containing *T. vaginalis* and the drugs except the decoction from *S. flavescens* in the medium was stood for 5

days. Ten μl media taken was subjected to the observation and count of the moving and immobilized parasites everyday for 5 days. The criterion for the distinction between moving and immobilized parasites was based on the observation above described. The test was repeated three times.

RESULTS

Preliminary study to exclude non-effective drugs

The results of the preliminary tests based on the observation for 5 hr (Table 1) showed that the extract of *A. annua*, *P. amurense* and *P. chinensis* was highly effective in immobilizing the parasites. These 3 kinds of extraction showed high efficacy against the parasites as early as 1 hr after the start of the exposure. Especially the extract of *A. annua* and *P. chinensis* were found to be extremely effective irrespective of the drug concentrations tested at 1 hr after the start of the exposure. Compared with the efficacy of the extracts from *A. annua* and *P. chinensis*, that from *P. amurense* remained less effective at lower concentrations of the extract until 3 hr after the start of the exposure. At 4 and 5 hr after the start, there was scarcely any differences among these 3 test materials.

The extracts of *C. monnieri* and *S. flavescens* were found to be much less effective if they had any efficacy (Table 1). At 5 hr after the start of the exposure, the extract from *C. monnieri* seemed to have somewhat effectiveness at 4- or 8-fold dilution. However, no efficacy was detected in the extract from *S. flavescens* under the present experimental conditions.

Thus, the present preliminary study showed that the extracts from all the plants examined except *S. flavescens* had, more or less, an effect immobilizing or killing *T. vaginalis in vitro* following the exposure for 5 hr or less. As the decoction from *S. flavescens* showed no efficacy, this was omitted in the subsequent study.

Examination for finding such drugs that immobilize the parasites completely at certain concentrations

The effects of the extracts from *A. annua*, *C. monnieri*, *P. amurense* and *P. chinensis* at comparatively low concentrations were examined for 5 days (Table 2).

The extracts from *A. annua* and *P. chinensis* were found to be highly effective, immobilizing 100% of the parasites at 64-fold dilution, 2 (*P. chinensis*) or 3 (*A. annua*) days after the start of the exposure and afterwards. No complete immobilization was seen under the presence of these extracts at 128-fold dilution or less

concentrations.

The extract from *C. monnieri* was shown to be less effective than those from *A. annua* and *P. chinensis*.

The efficacy of *P. amurense* decoction against *T. vaginalis* was nearly the same as that of *C. monnieri* during 3 days from the start of culture.

DISCUSSION

Infection with *T. vaginalis* was found wherever search was made (Faust *et al.*, 1970). In Japan, 5–10% female and 1–2% male adults were infected with the parasite though the percentage was decreasing (Yoshida, 1992). This sexually transmitted disease, severely harmful to female health, is still nowadays a problem of public-health importance in China. Though the infection rate varies from region to region, one third of females are presumably suffering from the infection with the parasite in some areas of China (Zhang, unpublished). Thus, unfortunately too many people have been infected with this parasite all over the world.

The curative efficacy of metronidazole (flagyl) against *T. vaginalis* is well-known. However, this drug is accompanied with such difficulties that it is rather expensive for many patients as a local standard and is not readily available because of transportation problems as mentioned in INTRODUCTION.

Another way to treat the patients is the use of extracts from medicinal plants. However, this also has a problem to be resolved. The curative efficacy of crude drugs from plants has been obscure and the incomplete treatment of the disease seems to be responsible for another transmission, producing chronic carriers who are very dangerous from the viewpoint of epidemiology.

The aim of the present investigation on the 5 species of Chinese herbal medicine against *T. vaginalis* was to select them scientifically for the development of the new antitrichomonal drugs available even in local areas in China. It is hoped that the topical application of such drugs to patients would eventually control the further transmission of the parasitic disease. Needless to say, such plant-derived drugs would have the possibility of playing an alternative role in the treatment of *T. vaginalis* patients when this parasite is found to be resistant to metronidazole in developed countries and areas.

In both the present preliminary and the subsequent investigation, the extracts of *A. annua* and *P. chinensis* were found to be highly effective against *T. vaginalis*, immobilizing 100% of the parasites under certain conditions. Though extract from *C. monnieri* is described as

a drug effective against the disease caused by *T. vaginalis* (Mitsuhashi, 1988; Namba, 1993a), the effectiveness was not high as far as the present study was concerned.

The extract from *P. amurense* was fairly effective in the test of the exposure for 5 hr while it was not so effective in that for 5 days. What was responsible for this is a matter for speculation now. One of the possibilities is that effective components in question are unstable.

Thus, the extracts described as a drug effective against protozoal and bacterial diseases were found to reduce the number of *T. vaginalis* *in vitro*. However, the extract from *S. flavescens*, thought to be useful in eliminating parasitic helminths (Mitsuhashi, 1988; Namba, 1993a) was not effective against the parasitic protozoa, *T. vaginalis* in the present study.

Based on the data so far obtained, the extracts from *A. annua* and *P. chinensis* are thought to be promising because they could suppress the movement of the parasites completely under certain conditions. However, the possible side effects of the extracts under the conditions still remain to be studied before clinical application.

In conclusion, the present research would be beneficial for the basic study leading to the development of new drugs from the wealth of Chinese medicinal plants which have been believed to be useful from the ancient times.

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