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Antiparasitic Effects of a Herb Extract from *Gentiana scabra* var *buergeri* on *Trichomonas vaginalis*

Yong-Suk Ryang†, Jee-Aee Im, Insik Kim, Yoon-Kyung Cho, Ho-Joong Sung, Ju-Youn Park, Duk-Young Min[1] and Ji-Yong Ha[2]

Department of Biomedical Laboratory Science and Institute of Health Science, College of Health Science, Yonsei University Wonju-City, Kangwon-do, 220-710, Korea, [1]Department of Parasitology, Hanyang University College of Medicine, Seoul, 133-791, Korea, [2]Department of Pathology, College of Oriental Medicine, Sangji University, Wonju-City, Kangwon-do, 220-702, Korea

We studied antitrichomoniasis with the extract of *Gentiana scabra* var *buergeri*, which may be effective in treating infectious diseases. The growth inhibition against *T. vaginalis* became optimal when the extract concentration was 0.7 mg/ml and the cells were seeded at a density of 3×10^5 per well. After incubation for 12, 24, 36, and 48 hrs, respectively, the number of cells were each 5×10^5, 1×10^5, 1×10^5, and none, respectively. Under the electron microscope, the experimental group showed that the nucleus, karyosomes, and chromatin were weaker than those in the control group. After incubating for 3 hrs, the cells were destroyed completely, and only a remnant remained. The hydrogenosomes disappeared almost. The vacuoles and autophagic vacuoles increased. The cells became regressive form.

Key Words: *Gentiana scabra* var *buergeri*, *Trichomonas vaginalis*, Antitrichomoniasis, Electron microscope

INTRODUCTION

Many herbs have antiparasitic effects and immunpotentiation and are used as therapeutic agents against parasitosis[2,4,10,11,13,14]. Quinine, which has therapeutic effects against malaria, is extracted from bark. It is used as an antimalaria agent[15]. The extracts of *Digenera simplex*, *Semen torreya*, and *Aspidium* are known as therapeutic agents against teaniasis[13]. In Oriental medicine, natural herbs are used as medicine against infectious diseases[2]. Recently, Soh *et al.* (1996)[14] reported the extract of *Coix lacryma* or *Coix lacryma* has antitoxoplasmosis and activaties macrophage. Oh *et al.* (1995)[11] demonstrated that the extract of *Artemisia annua* inhibited the proliferation of *Eimeria tenella*. Noh *et al.* (1995)[10] reported that rats treated with the extract of *Artemisia annua* and *Amaranthus mangostanus* showed significant inhibition to *Cryptosporidium parvum*.

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†Corresponding author: Yong-Suk Ryang
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Metronidazole (1-β-hydroxyethyl-2-methyl-5-nitroimidazole) was the treatment of choice for trichomonosis until metronidazole-resistant *T. vaginalis* appeared[5-8,12,15]. Experimental animals treated with metronidazole for a long period of time produced tumors, and metronidazole induced mutations in bacteria[8,12]. Now, a new treatment for trichomonoisis is needed.

In Korean Oriental medicine, *Gentiana scabra*, *Lonicera japonica* and *Taraxacum mongolocum* were used as therapeutic agents against bacterial, fungal, and parasitic diseases[2]. *G. scabra* which is a perennial herb, is involved in Gentianaceae and lives in several mountains in Korea[2]. It isn't well known as an agent of pharmacological and therapeutic effect against infectious diseases.

We studied whether the extract of *G. scabra* has a cytotoxic effect on *T. vaginalis*, induces morphological changes in worms, and would be used as a therapeutic agent.

MATERIALS AND METHODS

1. *T. vaginalis* culture

T. vaginalis Tri-4 was grown in Diamond's TYM medium (1957).

Table 1. Generation times of *Trichomonas vaginalis* cultured in TYM media

Number of Trophozoites \ Time (hr) after incubation	0 hr	12 hr	22 hr	42 hr	52 hr	62 hr
1 (1×10^5)	1	19	7	44	61	57
3 (3×10^5)	3	31	77	81	44	12
5 (5×10^5)	5	27	41	93	33	22
7 (7×10^5)	7	42	45	53	67	25
9 (9×10^5)	9	74	96	46	21	18
10 (10×10^5)	10	107	73	40	17	10

Table 2. Antiparasitic effects of a herb extract of *G. scabra* on the *T. vaginalis*[b)]

Dose of Herb extract[a)] \ Incubation Time	24 hrs incubated	Time after incubation**			
		12 hrs	24 hrs	36 hrs	48 hrs
Control	1.2×10^6	1.7×10^6	2.7×10^6	3.6×10^6	1.3×10^7
0.1 mg/ml	6×10^5	4×10^5	1×10^5	1.4×10^6	2×10^5
0.3 mg/ml	7×10^5	5×10^5	2×10^5	2×10^5	1×10^5
0.5 mg/ml	8×10^5	7×10^5	3×10^5	2×10^5	1×10^5
0.7 mg/ml***	$5\times10^{5***}$	$5\times10^{5***}$	$1\times10^{5***}$	$1\times10^{5***}$	0^{***}
1 mg/ml	7×10^5	8×10^5	5×10^5	1×10^5	0

[a)] The herb extracted by distilled water, and its dilution rate was limited in 0.2 mg/ml. [b)] *T. vaginalis* was seeded at a density of 3×10^5 per well. [c)] The experiment was repeated 5 times.

2. Extract of *G. scabra*

The trunk of *G. scabra* (200 g) was dissolved with 1000 ml 70% methanol (MERCK, Germany) in a 3000 ml flask, warmed, cooled, and filtered. The extract was made by lyophilization at -40℃. It was dissolved with 0.01 M Tris-HCl (pH 7.4) and filtered through a membrane filter (0.22 μm). The extract was diluted to the concentrations of 50 mg/ml, 40 mg/ml, 30 mg/ml, 20 mg/ml and 10 mg/ml and stored at 4℃.

3. Generation time of *T. vaginalis*

T. vaginalis was seeded at a density of 1×10^5, 3×10^5, 5×10^5, 7×10^5, 9×10^5, and 10×10^5, respectively, per well. Cells were cultured at 37℃ in a 5% CO_2 incubator for 12, 22, 42, 52, and 62 hrs, respectively. The number of cells was counted. The experiment was repeated 5 times and statistical significance was analyzed.

4. Cytotoxicity test

For the cytotoxicity test, *T. vaginalis* was seeded at a density of 1×10^5, 3×10^5, 5×10^5 and 7×10^5 per well. Cells were treated with an extract of *G. scabra*. Each concentration was 0.1 mg/ml, 0.3 mg/ml, 0.5 mg/ml, 0.7 mg/ml and 1 mg/ml, respectively. The cells were incubated at 37℃ in a 5% CO_2 incubator for 12, 24, 36, and 48 hrs, respectively. Cell survival was identified by trypan blue staining. The experiment was repeated by 5 times.

5. Electron microscopic observation of minute structure

The experiment was divided into a control group and an experimental group. The control group was treated with PBS and incubated for 1, 2, and 3 hrs, respectively. Thereafter, the cells were fixed with 3% glutaraldehyde at 4℃. The experimental group, which was treated with *G. scabra* (100 μl/ml), was incubated for 1, 2, and 3 hrs, respectively. Thereafter, these cells were fixed with 3% glutaraldehyde at 4℃, embedded, sectioned, stained with uranyl acetate and lead citrate, and observed with a transmission electron microscope.

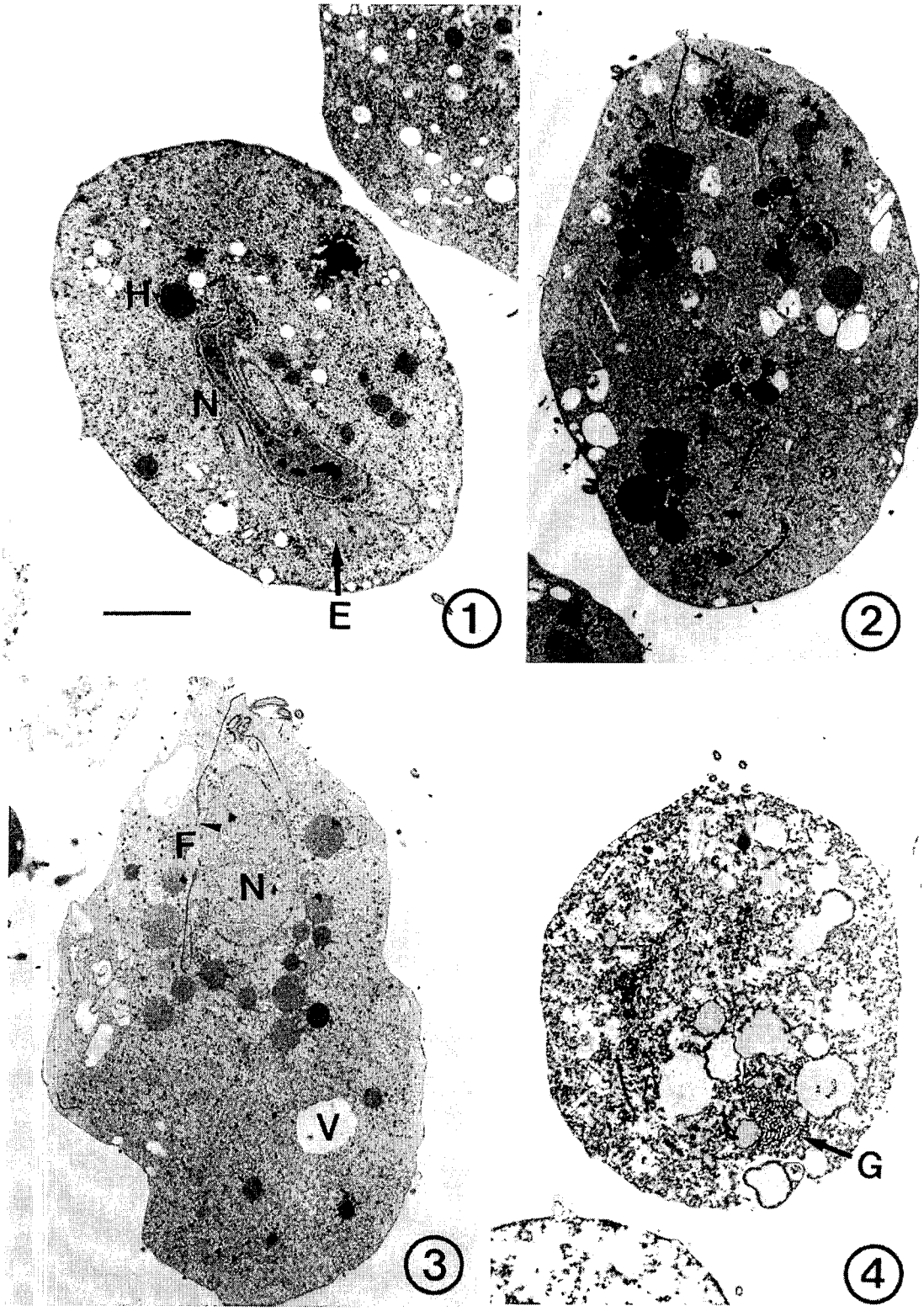

Fig. 1. Control, electron micrograph of a *Trichomonas vaginalis* from cultured for one hr.; N, nucleus; H, hydrogenosome (X 4000), bar, 2 μm

Fig. 2. Electron micrograph of a *T. vaginalis* by adding modulators with extract of *G. scabra* for one hr *in vitro* (X 3000).

Fig. 3. Control *T. vaginalis* from cultured for two hrs; F, flagellum; V, vacuole (X 3000)

Fig. 4. *T. vaginalis* treated with extract of *G. scabra* for two hrs; G, Golgi complex (X 3000)

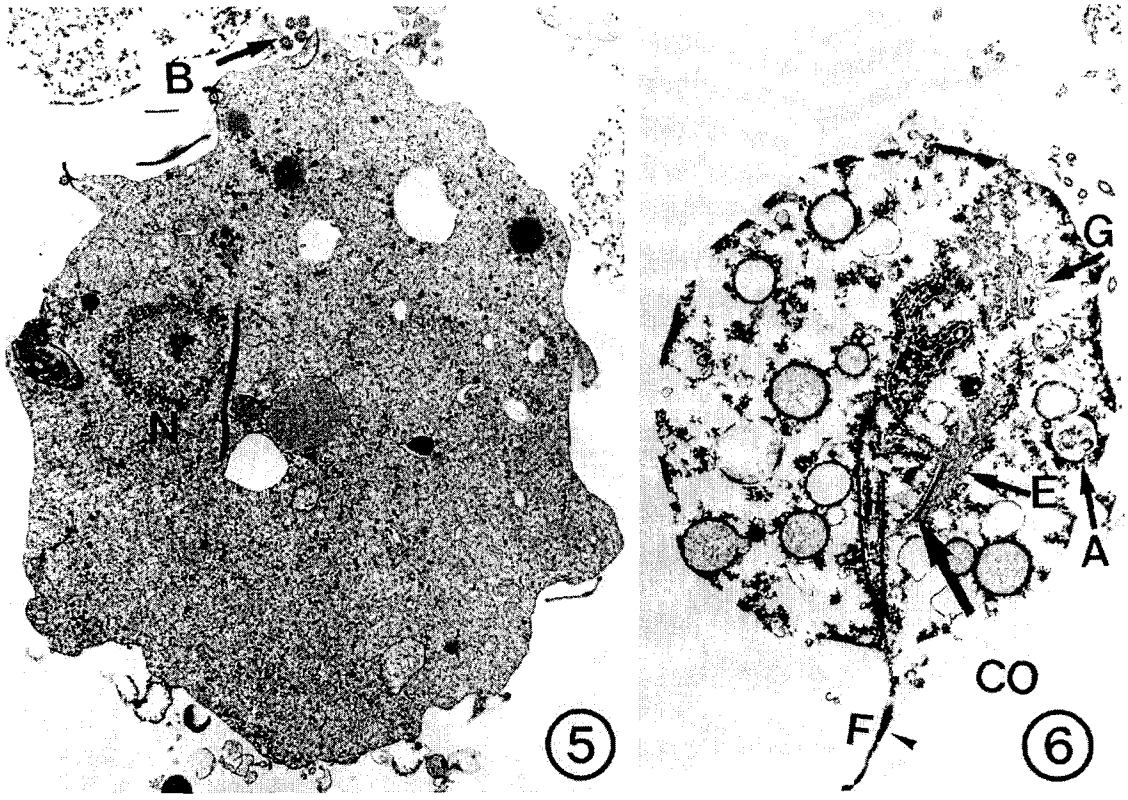

Fig. 5. Control *T. vaginalis* from cultured for three hrs; B, Basal bodies (X 3000)
Fig. 6. *T. vaginalis* treated with extract of *G. scarbra* for three hrs; A, Autophagic vacuole; E, Rough endoplasmic reticulum

RESULTS

1. Generation time of *T. vaginalis*

In the case of small number of the *T. vaginalis*, they increased slowly up to 48 hrs; thereafter, the number of cells decreased. In the case of the large number of *T. vaginalis*, they increased abruptly up to 12 hrs; thereafter, the cells decreased rapidly. The group that was seeded at a density of 3×10^5 per well showed the best survival curve (Table 1).

2. Cytotoxic effect against *T. vaginalis*

The group that was seeded at a density of 3×10^5 per well increased by 5×10^5 for 12 hrs after incubation. After the group was treated with *G. scabra* (0.7 mg/ml), it was incubated for 12, 24, 36, and 48 hrs, respectively. After incubation, the number of cells in group decreased by 5×10^5, 1×10^5, and 1×10^5, respectively, for each time (Table 1 & 2). The inhibiton of *T. vaginalis* did not completely concur with the concentration of *G. scabra*; however, the maximum inhibition of *T. vaginalis* was shown in the group which was seeded at a density of 3×10^5 per well and treated with *G. scabra* (0.7 mg/ml).

3. Electron microscopic observation

The control group treated with PBS for 1 hr appeared to have a normal morphology (Fig. 1). It was observed that the nucleus divided actively, karyosome was divided into two, chromatin was dense and regular, and the cell wall was apparent. The vacuoles and hydrogenosomes were appeared in the cytoplasm (Fig. 1). The experimental group treated with *G. scabra* for 1 hr had more heterochromatins, vacuoles, and hydrogenosomes than the control group. It had irregular morphology (Fig. 2). The control group treated with PBS for 2 hr showed a cell wall, and nucleus, chromatin was stained less than the control group treated with PBS for 1 hr. The vacuoles inflated, and the hydrogenosomes diffused in cells (Fig. 3). The experimental group treated with *G. scabra* for 2 hr showed almost no nucleus, and few karyosomes. The vacuoles were destroyed. It was observed that the Golgi complex in the experimental group was condensed (Fig. 4). The control group treated with

PBS for 3 hrs showed a nucleus and cytoplasm, which were more irregular than the control group treated with PBS for 2 hrs (Fig. 5). It was also observed that the nucleus and nuclear membrane disappeared. The nucleus was surrounded by diffused chromatin, and the karyosomes were weak (Fig. 5). The experimental group treated with *G. scabra* for 3 hrs was destroyed abruptly, and many vacuoles and unusual material appeared in the cytoplasm. The cell wall also was destroyed (Fig. 6). The shape of the nucleus was damaged, and only a remnant remained. It was observed that the cell had hydrogenosomes, autophagic vacuoles, and electron transucents. Because of the regressive changes in the cells, it is difficult to observe the internal structure of all of the cell (Fig. 6).

DISCUSSION

Trichomonosis, the most common non-viral sexually-transmitted vaginitis, is caused by *T. vaginalis*. The treatment for this disease is difficult because it often relapses[1]. Metronidazole is the treatment of choice for trichomonosis. The mechanism in action of metronidazole against trichomonosis, is the inhibition of DNA synthesis in cell division [3,5,9]. However, it was reported that *T. vaginalis* is resistant to metronidazole[12,15]. In Korea, it was reported that *T. mongolocum* and *G. scabra* were used as Oriental drugs to treat Trichomonosis[2], but the mechanism of these Oriental drugs on treatment of trichomonosis didn't identified. A major component of *G. scabra* is gentiopicrin $C_{16}H_{10}O_9$ (8~9%), and other components are alkaloid gentianine $C_{10}O_2N$, and gentianose $C_{18}H_{32}O_6$[2]. In Oriental medicine, *G. scabra* is used as a drug for bacterial infectious diseases such as cholangitis, duodenitis, urethritis, and conjunctivitis and parasitic infectious diseases such as ascariasis[2]. The therapeutic efficacy of *G. scabra* is, however, still unknown. We think *G. scabra* has an inhibitory effect against *T. vaginalis*. *T. vaginalis*, which was seeded at a density of 3×10^5 per well, grew well for 41 hr; thereafter, it decreased (Table 1). The experimental group, which was treated with an extract of *G. scabra* (0.7 mg/ml) showed excellent antitrichomonosis. The extract of *G. scabra* was crude extract. We suppose that if a crude extract is fractioned and becomes a pure substance, it will be an excellent therapeutic drug against antitrichomonosis.

In this present study using electron microscopy, the experimental group treated with *G. scabra* showed that hydrogenosomes, vacuoles and heterochromatins increased according to the treatment time, respectively. After being treated for 3 hrs, *T. vaginalis* was almost destroyed. Rosenkranz *et al.* (1975)[12] reported that *T. vaginalis* treated with metronidazole showed that free ribosomes increased, and polyribosomes decreased as observed by electron microscopy. It was indicated that DNA synthesis was inhibited, and cell division was relayed. Metronidazole blocks electrons such in H_2 in *T. vaginalis*[12]. As a result, metronidazole blocks cell division, which prohibits the interphase of the cell cycle (Fig. 1, 2, 5 & 6). Muller (1980)[9] demonstrated that hydrogenosomes were associated with anaerobic oxidation in pyruvate metabolism, and the destruction of hydrogenosomes followed cell death. Lahti *et al.* (1994)[5] reported that *T. vaginalis* in a medium without ferrous ions decreased in the number of hydrogenosomes and also in that of polysomes (Fig. 2 & 6). The later was surrounded with vacuoles (Fig. 2 & 6). Lindmark *et al.* (1973)[7] demonstrated that polysomes were associated with secreted protein. We suppose *G. scabra* blocks the very important function in cells, such as DNA synthesis, protein synthesis etc, but we don't know how *G. scabra* has an growth inhibiton of *T. vaginalis*. We also suppose that the results of cell membrane destruction and increasing autophagic vacuole by treatment of *G. scabra* are effect to protein synthesis in *T. vaginalis* (Fig. 2, 4 & 6).

Above all, we identified that an extract of *G. scabra* inhibited the growth of *T. vaginalis*. In further, the study will be focused on the effect of *G. scabra* against *T. vaginalis* on pharmacological mechanism, i.e. DNA, protein metabolism.

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