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Study the Effect Of *Cladophora glomerata* Algae Extract on the *Trichomonas vaginalis* Parasite

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Abstract

Trichomonas vaginalis is unicellular protozoan flagellate parasite that has only the trophozoites stage. It has no cystic stage in its life cycle. Vaginal swabs were collected from women attending Ibn Al-balady hospital, suffering from inflammation, itching and burning of the vagina, the samples transported to the laboratory after putting them in 5ml of Diamond modified media, the study was conducted on 80 female's mice in age 8 weeks and weight (25-35g) given 10^5 trophozoites/ml in the vagina for 10 days. After confirm infection the mice were given alcohol extract of *Cladophora glomerata* at two concentration 128 mg/ml and 256mg/ml. the best result of the decrease the number of trophozoites on the sixth day of treated group by concentration (128-256mg/ml), also metronidazole (Flagel group) the parasite killed at sixth day, the result showed varied histopathological changes in the uterus, vagina, testis in infected animals with *T. vaginalis*, included inflammation, cellular infiltration, secretion activity and hyperplasia of squamous epithelial cell.

Keywords: *Trichomonas vaginalis*, *Cladophora glomerata*, metronidazole

INTRODUCTION

Trichomonas vaginalis is protozoa and flagellate parasite the trophozoite consider diagnostic stage and infective stage can cause inflammation of vagina in women and urethritis in men, normal vaginal discharge was appeared clear or milky when it was dried on clothing [1]. Infrequently might notice white spots or a normal vaginal discharge what was thin and stringy looking [2]. The change of vaginal discharge can be clinical manifestation of vaginitis [3]. Intracellular and extracellular cytotoxic extracts of *Cladophora glomerate* shown some activity against bacteria, fungi and parasites [4,5]. Metronidazole is the treatment of *T. vaginalis* [6], with compound such as tinidazole and seconidazole [7]. In this study, the activity of *Cladophora glomerata* were studied against pathogenic *T. vaginalis*

MATERIALS AND METHODS

Patient and samples:

This study was carried out during the period from June 2017 to January of 2018, the samples of *T. vaginalis* were collected from women attending Ibn Al-balady hospital, they suffer from the symptoms of the vaginitis, itching and burning, a sterile cotton swab was used to collect the vaginal discharge from the posterior vaginal fornix [8]. Examined a sample of vaginal swab by rolling the swab on clean glass slide, left to dry at room temperature then fixed by using 100% absolute methanol for 30 seconds, left to dry again and stained with geimsa stain for 20 minutes [9]. Washing in tap water, dry it and examined under (100x).

Cultural methods

Broth culture of *T. vaginalis* is considered the "gold standard" for the diagnosis of trichomoniasis, culture technique by using diamond's modified medium [10], pH 6.6, 10^5 trophozoite/ml is the minimum inoculum size required for a positive result [11].Add 0.5 of fetal bovine serum to each 5ml of media. Incubated at 35℃ for 72h. The parasite was subculture in diamond modified media every 5 days for maintain the growth of the parasite.

Collection and diagnosis of Algae samples

The samples were collected according to (4) from the bottom of the Al-Najaf sea zone on by a plastic container, size 5 liters, washed with tap water to remove dirt and left to dry at room temperature, then grind with an electric mill and pressed in dry packaging. Then grinded and placed in the refrigerator at a temperature of 4^0C.untile they will be used.

Preparation of extract

The method of (12) was depended on in the preparation of the extracts of green algae *Cladophora glomerata*, the sample was placed in the Soxhlet and chloroform was added at a concentration of 99%). The extraction process was then carried out in the extraction apparatus for 4-5 hours until a colorless filter was obtained at a temperature of 60-50°C. the extract had been filtered with the filter paper (Whatman No.1). After that drained, the residual leachate had been incubated at 37^0C for 48 hr. to obtain the dry powder and stored it in the refrigerator until use. One gram of dry extract dissolve in 2 ml of alcohol to obtain 500mg /ml, it had been sterilized by 0.22µ filter papers, which was considered the standard solution, and was attended by 128mg/ml and 256mg/ml.

Preparation of laboratory animals

In this study, 80 mice of the white mice white swiss mice (males and female) , were obtained from national center for research and drug control, age between (5-12) weeks, weight (16-22) gm, were injected by1x105 tropho /ml in the vagina, after 48 hours, the swab had been taken from all mice and put on clean slide to be examined by light microscope under 10x, then 40x [13].

Infected mice were divided for 4 group:

Group 1: given (1 ml) from metronidazole orally at a single dose per day.

Group 2: given (1 ml) of the algae extract at 128mg/ml concentration orally at a single dose per day.

Group 3: given (1 ml) of the algae extract of 256mg/ml concentration orally at a single dose per day.

Group 4: given (1 ml) of phosphate buffer solution orally.

Histological examination

The mice were killed and extracted the organs (testis and vagina). It is carried by a string of successive processes

according to the method describer by [14] and staining by Hematoxylin and Eosin [15]. Evaluation the efficiency of algae extract by counting the number of trophozoite in treated group using hemocytometer.

Percent inhibition = a^{a-b} x 100 growth.

a: mean the number of trophozoite in control mice.

b: mean the number of trophozoite in treatment mice [16].

RESULTS AND DISCUSSION

Table (1) shows that the number of trophozoites decrease in the treated group with algae extract at the sixth day, because the algae extract has an effect on bacteria isolated from wound and burns (in vitro). The *Cladophora glomerata* has been identified as a rich and renewable source of biologically active compounds that may be useful as therapeutic agents with antioxidant, anticancer and antibacterial activity such as: myristic acid, methyl ester ; ppropiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl); dodecanoic acid, methyl ester; 9,12,15-Octadecatrienoic acid, (z,z,z); propanoic acid, 2-methyl-, methyl ester and the other compounds such as imidazole, 2-amino-5-[(2-carboxy)vinyl]; 2,4-di-tert-butylphenol; dihydroactinidiolide and butane, 1-ethoxy [17].

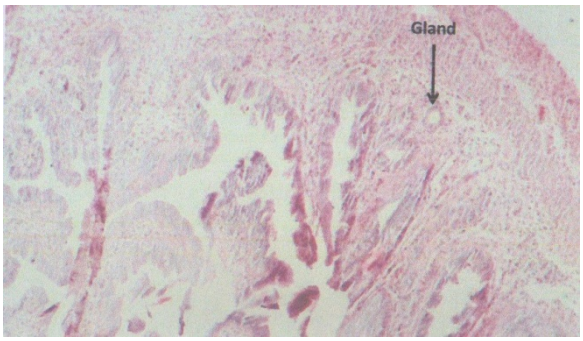

Fig. (1): The section of the uterus in animal (negative control group) showing normal structures appearance lined by columnar H & E (400x)

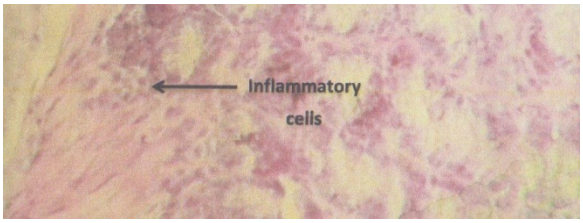

Fig (2): Section of vagina of treated group with algae extract (128mg/ml) showed inflammatory cells in these tissues of mice H&E (400x)

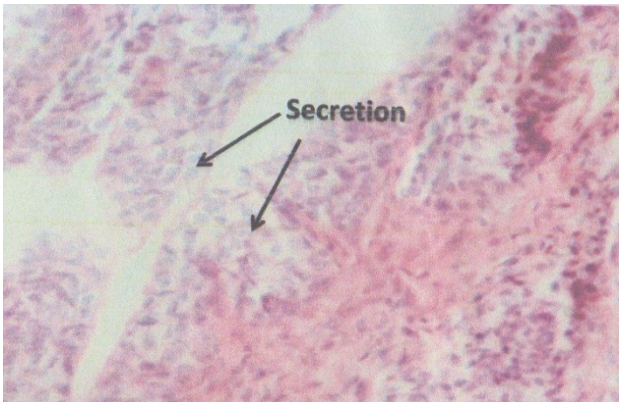

Fig. (3): Section of vagina of treated group with algae extract (256mg/ml) showed secretion in these tissues of mice H&E (400x)

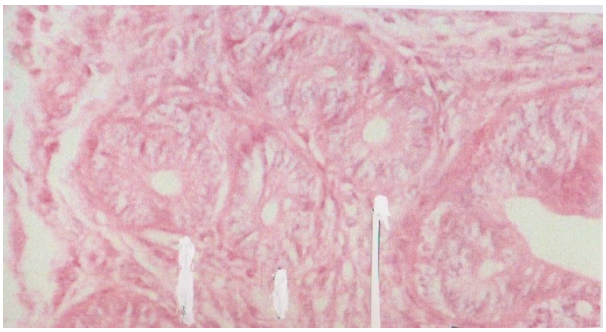

Fig. (4): The section of the uterus in positive control group showing Hyperplasia of lining epithelial cells H&E (400x)

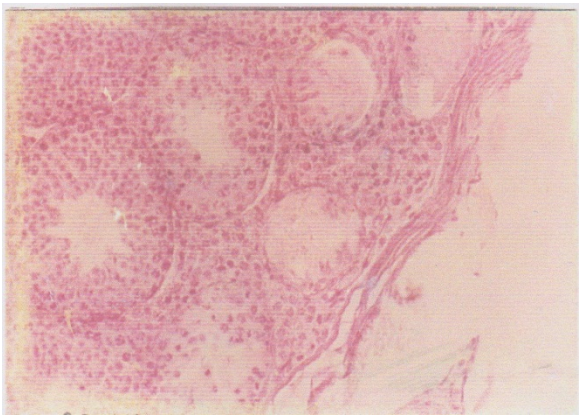

Fig. (5): Section of testis of treated group with algae extract (128mg/ml) showed prominent secretion activity in the cells H&E (400x)

Table (1): The number of trophozoites/ml in different group

	Days					
	1	**2**	**3**	**4**	**5**	**6**
Metronidazole	12.16±0.9	10.9±0.9	6.0±1.1	3.6±1.2	0.00	0.00
Control	17.6±.1.9	19.0±1.3	20.0±1.5	22.0±0.9	25.5±0.8	26.6±0.7
128mg/ml extract of algae	17.6±1.9	14.9±0.8	11.9±0.9	6.6±1.1	3.6±1.2	2.7±1.4
256mg/ml extract of algae	14.9±1.5	12.7±1.2	7.5±1.3	6.0±1.1	3.5±1.2	2.1±1.2

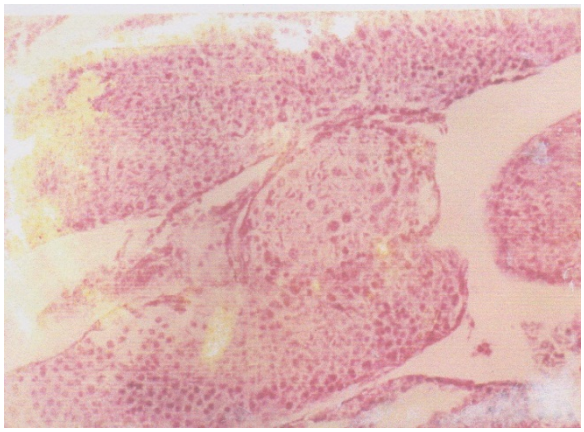

Fig. (6): Section of testis of treated group with algae extract (256 mg/ml) showed Odema H&E (400x)

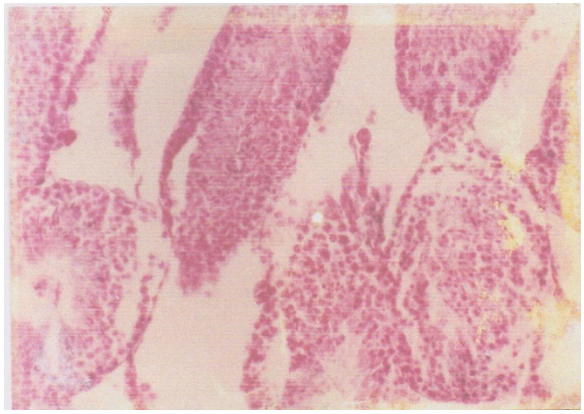

Fig. (7): Section of testis of treated group with algae extract (256 mg/ml) showed necrosis in the cell H&E (400x)

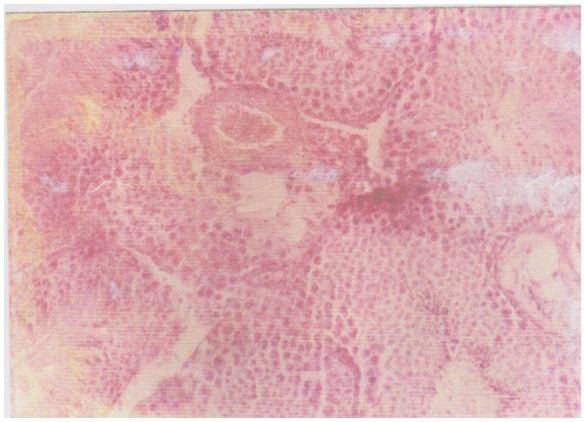

Fig. (8): Section of testis of positive control group showing Odema and congestion in the blood vessicles H&E (400x)

The vagina is lined with non-karatinizing squamous epithelium, (8-12cm) long, and it is a fibro muscular sheath like structure linking the external genitals with the uterus [18]. In current study the infected by *T. vaginalis* caused infiltration of inflammatory cells, and this related to vaginitis, endometritis, and this led to activate inflammatory responses in the mucosal genital tract. Also, *T. vaginalis* carries viruses and other parasites, such as mycoplasma and gardenella, causing chronic mucosal damage and an inflammatory reaction which gives rise to severe consequences in reproductive outcomes. The ability of *T. vaginalis* to avoid the immunity of the host may be due to the presence of adhesion protein, lipophosphoglycan and cysteine protease molecules [19]. The cysteine protease (CP) which recreated from *T. vaginalis* causes destruction of vaginal cell of host and stimulate apoptosis in human vaginal epithelial cells of apoptosis, may have significant implications for therapeutic intervention [20], the hyperplasia which occurred in squamous epithelium of vagina in current study may be caused by presence of *T.vaginalis*, which caused increased in glucose[21] , this increase will lead to increase the glycogen in vagina and this led to increase the estrogen hormone and caused vaginal hyperplasia [22].

In vitro

The result of the tests for the extract of algae in decrease the trophozoites of *T.vaginalis* showed in current study, the concentration of 128mg/ml killed 350,000 troph/ml and the 256mg/ml killed 400,000 troph/ml.

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