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Epidemiology Study of *Trichomonas Vaginalis* in Babylon Province and the Efficiency of *Mentha Spicata* Leafs Extracts in Vivo

Dr. Maher Ali Al – Quraishi
Department of Biology - College of Science - University of Babylon/ Iraq*
Email: alquraishi_maher@yahoo.com

Abstract
The current study was conducted in the period from November 2012 to June 2013 to investigate the epidemiology of trichomoniasis in Babylon province, the total number of examined urine samples was **600** for different ages in addition to 197 vaginal swab samples collected from hospitals, medical centers, and special clinics. The samples were examined by direct smear method and wet preparation method in hospital and medical centers laboratories and the advanced parasites laboratory in the college of the Science / Babylon Uni. This study include the relation of age, sex with the infection percentage of trichomoniasis , The result shows the total infection rate for female 7.38% and male 4.2% in urine in urban, lower than infection rate for female 12.16% and male 5.09% in urine samples in rural. the total infection rate in urban for vaginal swab 11.25% and higher in rural 16.23%.The effect of hot water extract is more efficient on *T. vaginalis* infections, we notice al animals recover in 40% hot water extract compared with cold water extract 3 animals recover in 40% con. and 3 animals recover in 40% con. of alcoholic extract.
Keywords: T. vaginalis , menthe spicata

Introduction:

Trichomonas vaginalis is a sexually transmitted disease (STD), although transmission by other routes (such as soiled towels) has been documented (Ryu *et al*., 2002 ;Mendoza-lo'pez *et al*., 2000). There is no cyst in the life cycle, so transmission is via the trophozoite stage. Most people infected with trichomoniasis are asymptomatic. Symptomatic infections are characterized by a white discharge from the genital tract and itching. Diagnosis depends on finding trophozoite in secretions of the genital tract from men or women. In cases where the numbers of organisms are very low, the trophozoite can be cultured to increase their numbers (Schwebke, and Hook, 2003;Weise and Patel, 2000).

T. vaginalis is a flagellated single cell eukaryote with a relatively simple lifecycle, divides by simple binary fission in its human host a closely related relative *Tritrichomonas foetus* causes commercially important reproductive tract and fetal infections in cattle. *T. vaginalis* carries the distinction of being the only truly sexually transmitted parasitic infection in humans (Shaio *et al* .,1997). It is very successful as a pathogen causing roughly the same number of STDs as *Chlamydia trachomatis*, the most prevalent sexually transmitted bacterial pathogen. In the U.S. there are an estimated 3-5 million new cases of trichomoniasis every year with an infected pool of approximately 20 million individuals (Schwebke and Hook, 2003). Worldwide the prevalence of *T. vaginalis* varies from 2% to greater than 50% depending on region, country, gender and demographics of the population specifically evaluated. *T. vaginalis* is highly adapted to the human urogenital tract and is never found in stool specimens. The unique adaptation of *T. vaginalis* to the urogenital tract allows it to be easily identified in urogenital tract clinical specimens without concern about other parasite species. *T. vaginalis* thrives in the microaerophilic environment of the vaginal mucosa. To live in the low oxygen tension it utilizes an organelle called a hydrogenosome to generate ATP. *T. vaginalis* lack mitochondria that generate ATP for oxygen-dependent eukaryotes. Instead the hydrogenosome generates ATP utilizing a pathway similar to mitochondria except that the final electron acceptor is hydrogen rather than oxygen, generating hydrogen gas as a byproduct of metabolism. The hydrogenosome is also the Achille's heel of *T. vaginalis* as it metabolizes the 5-nitroimidazole antibiotics Metronidazole and tinidazole into toxic anion radicals that kill the parasite (Marquardt *et al.,* 2003).

Mentha spicata has high traditional medicinal value as it is one of the important constituents of Ayurveda, Homeopathy and Siddha systems of medicine. Mentha can be used for common cold, cough, sinusitis, fever, bronchitis, nausea, vomiting, indigestion, intestinal colic and loss of appetite (Starburck, 2001). It can have a calming effect when used for insomnia or massages. Essential oil of Spearmint was found to have some antimicrobial activity (Hussain *et al*., 2010). It is also a safe and effective therapeutic option for the treatment of chemotherapy-induced nausea and emesis in patients (Najaran *et al*., 2013). Spearmint (*M. spicata* L.) is widely used as a source of essential oils for flavoring agents, and more recently it has been used as a valuable source of the potent antioxidant rosmarinic acid for the neutraceutical and cosmetic industries (Shetty, 2001). Rosmarinic acid has earned the reputation as a molecule of interest owing to its multiple biological

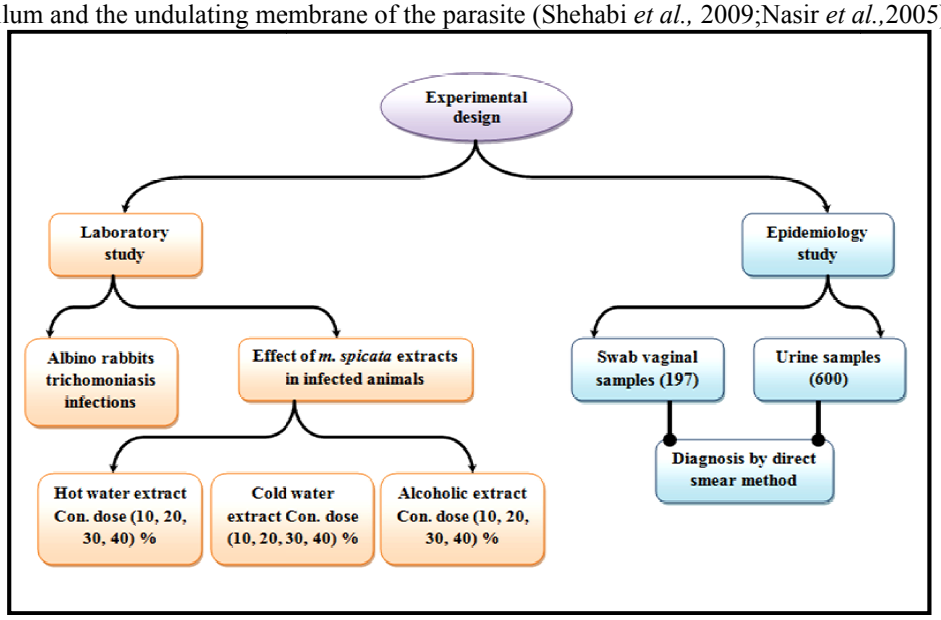
activities against inflammatory lung diseases, autoimmune arthritis, heart disease and suppression of autoimmune rejection in human skin transplant patients as well as its multipurpose activities against reverse transcriptase, integrase and RNase H in nHIV infections (Sanbongi *et al*., 2003; Hooker *et al.,* 2001). Therefore interest in cultivating a quantifiable natural source of this potent and versatile antioxidant has become paramount.

Aim of the study:

In this research we estimate the epidemiology of *T. vaginalis* infections in Babylon province hospitals and compare the infections rate between rural and urban areas also study the effect of alcoholic and cold, hot water extracts of *m. spicata* (spearmint) leaf in different concentrations on the infection of *T. vaginalis* in laboratory albino rabbits.

Materials and Methods:

A total of 600 samples were collected from male urine and 200 samples from female vaginal swab from November 2012 to June 2013 from Al-Hilla hospital, private laboratories, in Babylon province, some information was taken from patients such as name, address. Two methods used for examination, wet mount preparation method, one drop from deposit materials butting on clean sterilized slide and use cover slip to get a clear vision and examine in 40x, 100x Identification of the parasite by its motile and size and taking a smear from the sample on clean sterilized slide and fixed it by passing the slide on a flame, then use a several drops from a Giemsa stain for 5 min. and wash the slide by Distilled water. The staining method helps us to distinguish the flagellum and the undulating membrane of the parasite (Shehabi *et al.,* 2009;Nasir *et al.,*2005) .



Preparation of alcoholic extract of *m. spicata* (spearmint):

The collected plant were washed with water and separated from undesirable materials or plants or plant parts. They were partially dried by air and then heated in an oven at bellow 40ºC for two days to be fully dried. The fully dried leaves were then grinded to make them powder by the help of a suitable grinder. Then the powders were dissolved in methanol (80%) and kept for a period of 2 days accompanying occasional shaking and stirring. The whole mixture was then undergone a coarse filtration by a piece of clean, white cotton material followed by a second filtration through whatman filter paper. The filtrate obtained was evaporated by rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and 65ºC temperature. It rendered a gummy concentrate of chocolate black color that was designated as methanol extract of *M. Spicata* (MEMS). The crude methanol extract was finally dried by freeze drier and preserved. (Yousuf *et al.,* 2013).

Preparation of hot water of *m. spicata* (spearmint) extract:

150 g of *M. spicata* dried leaves were boiled in 3 L distilled water for 2 h. Then solution was filtered and dried by evaporation. The extract was dissolved in RPMI-1640 and filtered by 0.2 µm filter and stored at -20°C until use in experiments. The extract was diluted in culture medium to prepare the required concentrations before use (Hajighasemi *et al*.,2011).

Laboratory animals:

80 albino rabbit weight 5 ± 0.5 kg divided to 4 groups each group include 20 animals divided to 5 animals for each concentration of extracts were put in iron cages and supplied with especial food in house keeper of Science College /Babylon University and examined daily to confirm the infections of the animals, all animals infected with trophozoite stage by using saturated cotton then we examined the vaginal of all animals by Direct smear method and after we checked the present of infection for all animals.

Animals experimental:

After the trichomoniasis infection occurs in all female rabbits by using infected white discharge to made the infections of rabbits, then treated with hot and cold and Alcoholic spearmint extract under anesthesia, All animals' dosage the extracts by using saturated cotton with different concentrations twice a day as follows:

1. First group dosed with 10,20,30,40 mg/kg alcoholic extract.
2. Second group dosed with 10,20,30,40 mg/kg cold water extract.
3. Third group dosed with 10,20,30,40 mg/kg hot water extract.
4. Fourth group (control) dosed with normal saline 0.85%.

Statistical analysis:

Statistical analysis of the results was done by using t-test, $p<0.05$ as the lowest limit significance, **(spss).**

Result:

1- Epidemiology study

Table (1) and figure (1), shows total examined and infected urine samples for male and female patients according to ages in urban (Babylon province) , the total percentage rate for female was 7.38% higher than male 4.2%, the maximum infection percentage for male was 18.7% for ages (20-29) year and the minimum percentage was 3.8% for ages (30-39) year, while no infection recorded for ages (5-10) , (11-19) , (40-50) year.

The maximum infection percentage for female was 20% for ages (20-29) year and the minimum percentage was 2.2% for ages (40-50) year, while no infection recorded for ages (5-10). The statistical analysis result shows significant difference in infection rate for different ages.

Table (1): percentage rate of *Trichomonas vaginalis* prevalence in urine samples for male and female in urban.

Male				Female		
Age(year)	**No. specimens**	**No.of infected**	**%**	**No. specimens**	**No.of infected**	**%**
5-10	25	-	-	20	-	-
11-19	27	-	-	25	1	4
20-29	16	3	18.7	45	9	20
30-39	26	1	3.8	41	2	4.87
40-50	25	-	-	45	1	2.2
total	**119**	**5**	**4.2**	**176**	**13**	**7.38**

Tc=2.303 Tt=2.132 $p<0.05$ significant differences

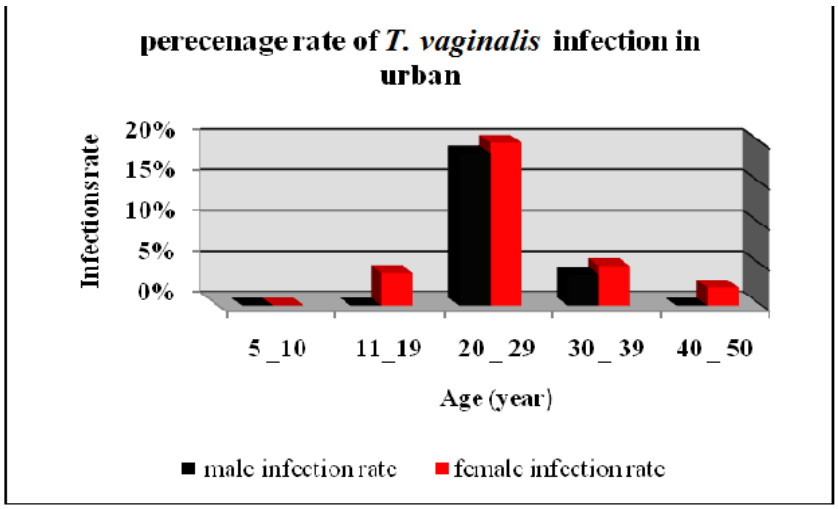


Figure (1): percentage rate of prevalence of *Trichomonas vaginalis* in urine samples for male and female in urban (Babylon province).

Table (2) and figure (2), shows total examined and infected urine samples for male and female patients according to ages in rural for Babylon province, the total percentage rate for female was 12.16% higher than male 5.09%, the maximum infection percentage for male was 13.1% for ages (20-29) year and the minimum percentage was 2.6% for ages (11-19) year, while no infection recorded for ages (5-10) year.

The maximum infection percentage for female was 22.9% for ages (20-29) year and the minimum percentage was 2.94% for ages (40-50) year, while no infection recorded for ages (5-10). The statistical analysis result shows significant difference in infection rate for different ages.

Table (2): percentage rate of *Trichomonas vaginalis* prevalence in urine samples for male and female in rural.

	Male			Female		
Age(year)	**No. specimens**	**No.of infected**	**%**	**No. specimens**	**No.of infected**	**%**
5-10	33	-	-	20	-	-
11-19	38	1	2.6	24	4	16.6
20-29	38	5	13.1	48	11	22.9
30-39	26	1	3.84	22	2	9.09
40-50	22	1	4.5	34	1	2.94
total	**157**	**8**	**5.09**	**148**	**18**	**12.16**

Tc=2.799 p<0.05 significant differences
Tt=2.132

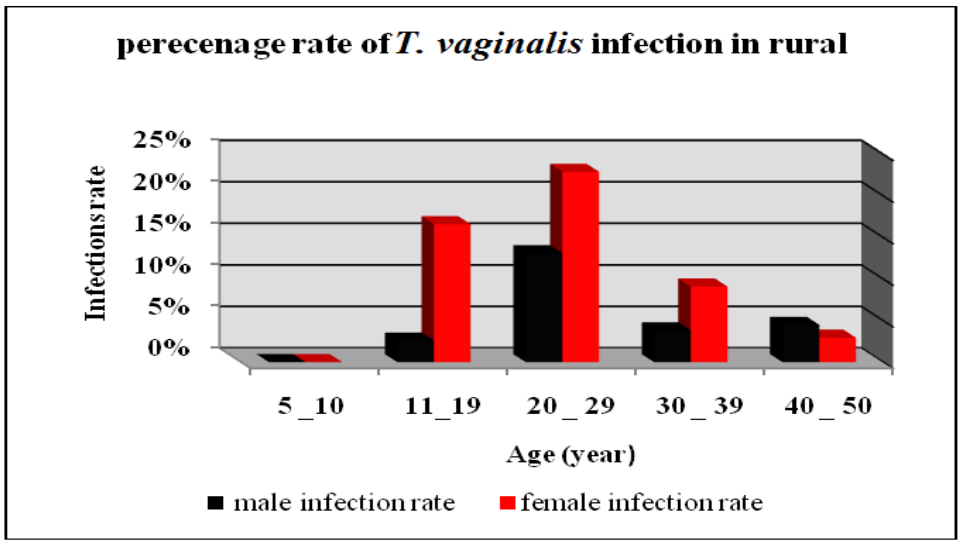


Figure (2): percentage rate of prevalence of *Trichomonas vaginalis* in urine samples for male and female in rural for (Babylon province).

Table (3) and figure (3), shows the total rate of trichomoniasis infections in vaginal swab in rural 16.23% higher than infection rate in urban 11.25% , the maximum infection percentage in urban was 16.6% for ages (20-29) year and the minimum was 10% for ages (30-39) year, while no infection recorded for ages (5-10), (40-50) year.

The maximum infection percentage in rural was 28.12% for ages (20-29) year and the minimum was 8.69% for ages (40-50) year, while no infection recorded for ages (5-10) year. The statistical analysis result shows significant difference in infection rate for different ages.

Table (3): percentage rate of *Trichomonas vaginalis* prevalence in vaginal swab samples in urban and rural.

urban				rural		
Age(year)	No. specimens	No.of infected	%	No. specimens	No.of infected	%
5-10	8	-	-	10	-	-
11-19	15	2	13.3	30	5	16.6
20-29	30	5	16.6	32	9	28.12
30-39	10	2	10	22	3	13.6
40-50	17	-	-	23	2	8.69
total	80	9	11.25	117	19	16.23

Tc=1.804 t=2.426 p<0.05 significant differences
Tt=2.132

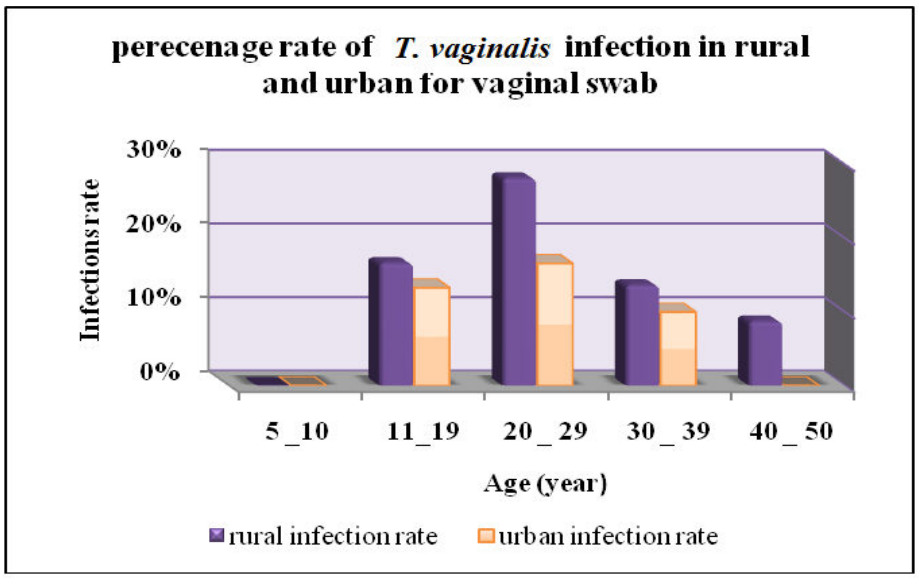


Figure (3): percentage rate of prevalence of *Trichomonas vaginalis* in vaginal swab samples in urban and rural (Babylon province).

Laboratory experimental:

In this study 5groups of albino rabbits used to discover the effect of alcoholic extract of *mentha spicata* on the infected animals, table (4) and figure (4) shows first group (5 animals) dosed with 10% alcoholic extract and no recovery recorded in this concentration, second group dosed with 20% alcoholic extract and one animal recover in third and fourth day, third group dosed with 30% alcoholic extract and one animal recover in 2ed day and two in third day and treatment stopped in forth day because Inflammations occurs , fourth group dosed with 40% alcoholic extract and three animals recover in third day and treatment stopped in forth day because Inflammations occurs.

Table(4):the effect of difference concentration of alcoholic extract of *mentha spicata* on infected albino rabbit.

days	G1 (10%)	G2 (20%)	G3 (30%)	G4(40%)	notes
1	0	0	0	1	
2	0	0	1	2	
3	0	1	2	3	
4	0	1	-*	-*	*stopped treatment Inflammations occurs

Tc=1.804 $p<0.05$ significant differences
Tt=2.132

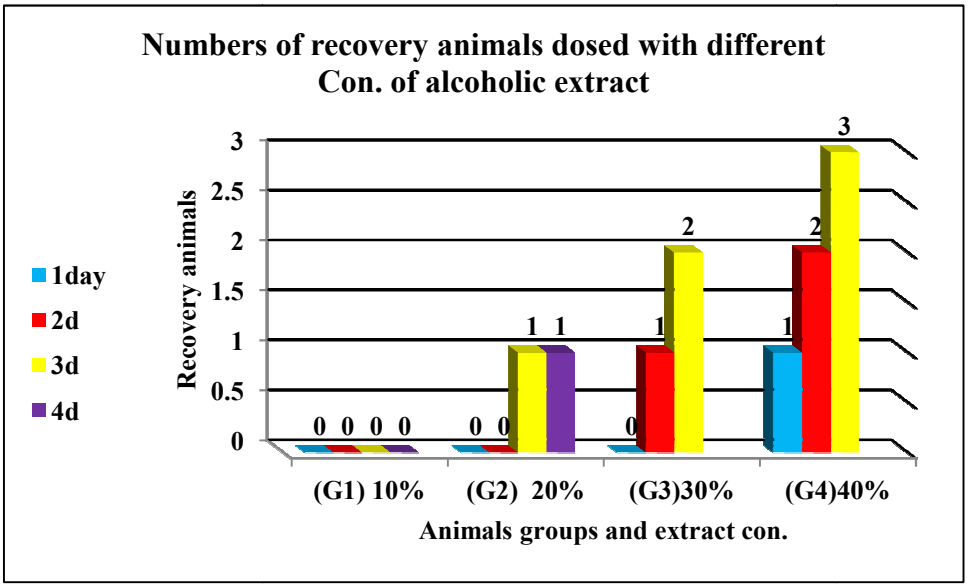


Figure (4): numbers of recovery animals dosed with different concentration of alcoholic extract of *mentha spicata* .

Table (5) and figure (5) shows the effect of cold water extract in infected animals in four groups, first group (5 animals) dosed with 10% cold water extract and one animal recover recorded in this concentration in 7th day of treatment, second group dosed with 20% cold water extract and one animal recover in 5th day and two recover in 6th and 7th day, third group dosed with 30% cold water extract and three animals recover in 7th day and the fourth group recorded four animals in the 7th day, and without any side effect for all animals.

Table (5):the effect of difference concentration of cold water extract of *mentha spicata* on infected albino rabbit.

days	G1 (10%)	G2 (20%)	G3 (30%)	G4(40%)	notes
1	0	0	0	0	-
2	0	0	0	0	-
3	0	0	0	0	-
4	0	0	2	1	-
5	0	1	2	3	-
6	0	2	3	3	-
7	1	2	3	4	-

Tc=3.627 p<0.05 significant differences
Tt=2.132

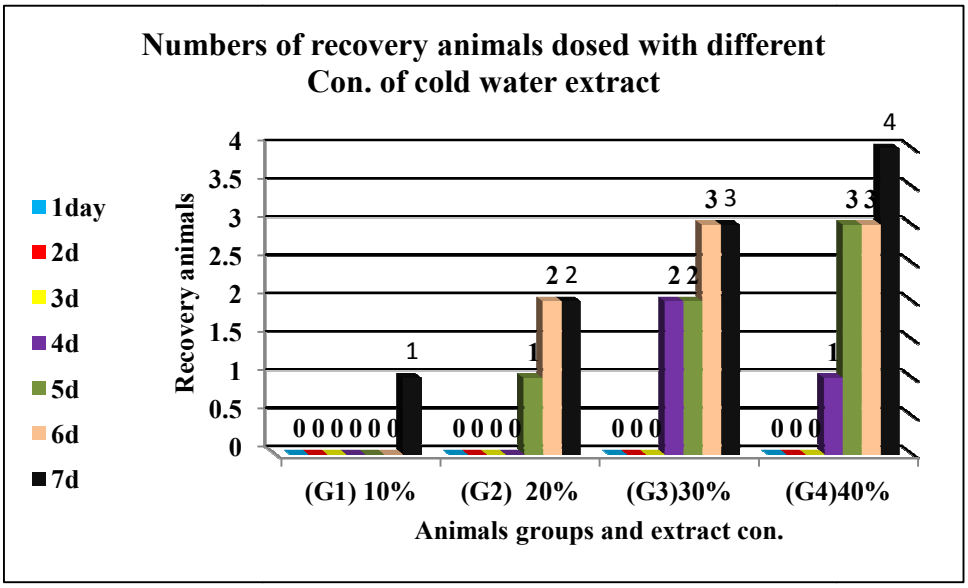


Figure (5): numbers of recovery animals dosed with different concentration of cold water extract of *mentha spicata* .

Table (6) and figure (6) shows the effect of hot water extract in infected animals in four groups, first group (5 animals) dosed with 10% hot water extract and one recover animal recorded in this concentration in 6th day of treatment, second group dosed with 20% hot water extract and two animals recover in 6th day, third group dosed with 30% hot water extract and three animals recover in 6th day and the fourth group recorded five animals in the 6th day, and without any side effect for all animals, figure (7) shows trophozoite stage of *T. vaginalis* stained by blue Methylene stain.

Table (6): the effect of difference concentration of hot water extract of *mentha spicata* on infected albino rabbit.

days	G1 (10%)	G2 (20%)	G3 (30%)	G4(40%)	notes
1	0	0	0	1	-
2	0	0	1	2	-
3	0	0	1	2	-
4	0	1	2	3	-
5	0	2	3	4	-
6	1	2	3	5	-

Tc=6.139 Tt=2.132 p<0.05 significant differences

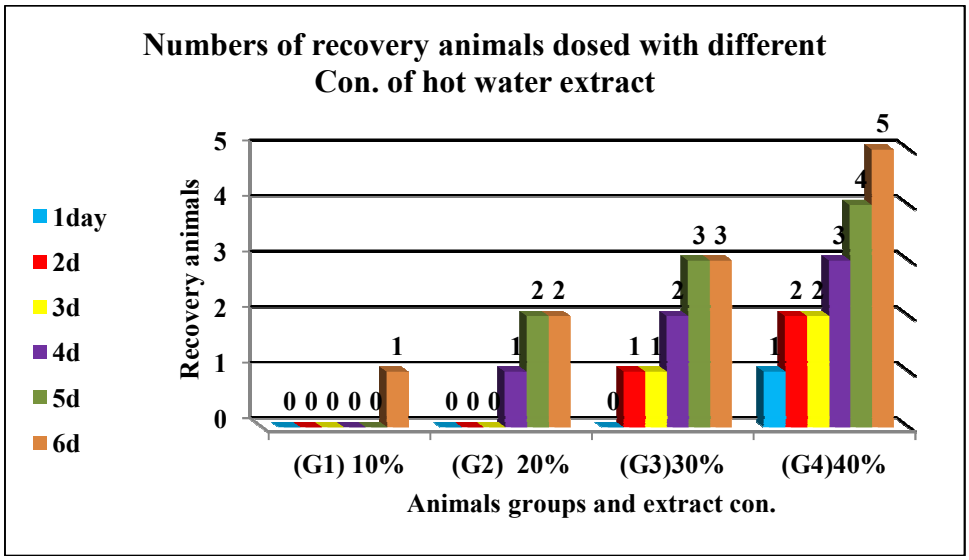


Figure (6): numbers of recovery animals dosed with different concentration of hot water extract of *mentha spicata* .

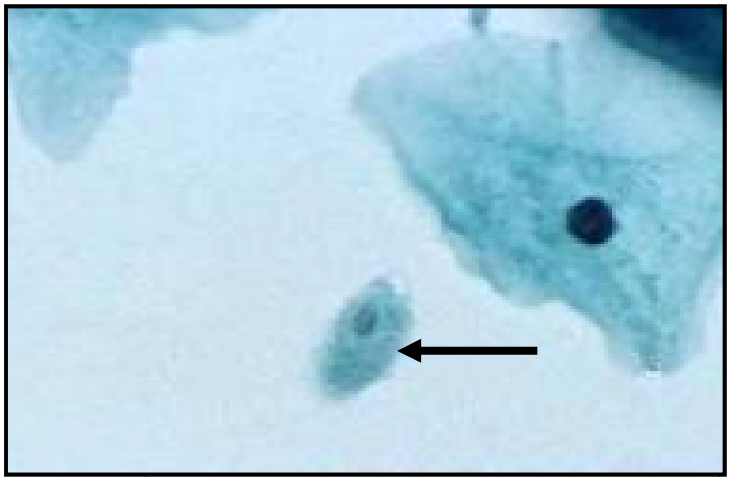


Figure (7): trophozoite stage of *T. vaginalis* in urine samples stained by Methylene blue stain, (40x).

Discussion:

Worldwide, *T. vaginalis* causes approximately 180 million new infections per year, making it the most prevalent nonviral sexually transmitted disease (STD) agent (Madico *et al*., 1998; Petrin *et al*., 1998; Kengne *et al.,*1994). Infections in women can cause vaginitis, urethritis, and cervicitis (Riley *et al.,* 1992). and complications include premature labor, lowbirth- weight offspring, and postabortion or posthysterectomy infection (Shaio *et al*.,1997). It has been estimated that 10 to 50% of *T. vaginalis* infections in women are asymptomatic (Burstein and Zenilman ,1999). and in men the proportion may even be higher. This parasite has also been implicated as a cofactor in the transmission of the human immunodeficiency virus and other non ulcerative STD agents.

In this study we found the infections rate of trichomoniasis in rural for female 12.16% higher than rate infections in urban 7.38% as well as for male rate infection in rural 5.09% while in urban was 4.2% , maybe these results due to differences between habitat conditions, economic level, environment contamination, education level, all these conditions may involved in cause the infection is higher in rural, and these results resemble other studies whose conclude that *T. vaginalis* infections be high incidence in poor health areas (Smith,2008; Sutherest, 2001). Al-Zabady (2004) Referred to the percentage of infections in rural 20.4% higher than infection rate in urban 19.9% , resemble to our result as in Table (3) and figure (3), shows the total rate of trichomoniasis infections in vaginal swab in rural 16.23% higher than infection rate in urban 11.25% , the maximum infection percentage in urban was 16.6% for ages (20-29) year and the minimum was 10% for ages (30-39) year and The maximum infection percentage in rural was 28.12% for ages (20-29) year and the

minimum was 8.69% for ages (40-50) year.

In our results the maximum infection percentage for male was 18.7% for ages (20-29) year and the minimum percentage was 3.8% for ages (30-39) year, while no infection recorded for ages (5-10) , (11-19) , (40-50) year. The maximum infection percentage for female was 20% for ages (20-29) year and the minimum percentage was 2.2% for ages (40-50) year, while no infection recorded for ages (5-10). and these results resemble the study of (Uneke *et al*.,2007). showed that the prevalence of infection was the highest 22.8% in age 26-30 year and the lowest rate 18.8% in age >40 year and the study of (Al-Hindi and Lubbud, 2006). showed the percentage rate infection in pregnant women 18.2% of 423 samples and the infection was high 22.9% in the age 21-30 year and in age 31-40 year 20.1% .

Mentha, a member of the Labiatae family is originated from Eastern Asia. Among the two major forms, namely *M. piperita* L. and *M. spicata* L , *M. spicata* is locally known as 'Pudina' in Bangladesh. Its English name is Spearmint which is 30–100 cm long and is characterized by its strong odor (Nadkarni, 2002; Kritikar and Basu ,1975). It has smooth or gray haired leaves and its flowers are pale blue and collected at the edges of the branches as a long and narrow spike. It contains volatile oil, carvone, limonene, cis-carveol, 1,8 cineol , cis-dihydrocarvone , carvyl acetate, cis-sabinene hydrate of which carvone is the most important constituent of *M. spicata* (Baser, 1993).

In our study we use three types of *M. spicata* extracts for treatment trichomoniasis infection in vivo , alcoholic extract was less effect in recover the infected animals cause in 3 recover animals in fourth day and inflammation occurs in animals vaginal and the treatment was stopped, while in cold water extract four animals recover in 7th day in group dosed with 40% table (5) , while the effect of hot water extract was the higher in recover animals rate as in Table (6) and figure (6), showed the effect of hot water extract in infected animals in four groups, first group (5 animals) dosed with 10% hot water extract and one recover animal recorded in this concentration in 6th day of treatment, second group dosed with 20% hot water extract and two animals recover in 6th day, third group dosed with 30% hot water extract and three animals recover in 6th day and the fourth group recorded five animals in the 6th day, and without any side effect for all animals, may be the cause of these results due to important chemical materials like tannins , phenolic compounds which had very important rules in disruption the cell wall of microorganisms, in the study of (Yousuf *et al.,* 2013). appeared that The aqueous extract of leaves of *M. spicata* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 100 mg/ml, in shorter time as compared to reference drug Piperazine citrate, Phytochemical analysis of the crude extracts revealed the presence of tannins among the other chemical constituent within them. Tannins were shown to produce anthelmintic activities.

Chemically tannins are polyphenolic compounds , Some synthetic phenolic anthelmintic e.g. niclosamide, oxyclozanide, bithionol etc., are reported to interfere with energy generation in helminthes parasites by uncoupling oxidative phosphorylation It is possible that tannins contained in the aqueous extract of leaves of *M. spicata* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tracts of host animal or glycoprotein on thecuticle of the parasite and may cause death (Thomson and Geary, 1995).

The traditional medicines hold a great promise as source of easily available effective anthelmintic agents to the people, particularly in developing countries, including in India. It is in this context that the people consumed several plants or plant derived preparation to cure helminthic infections (**Satyavati, 1990).**

The origin of many effective drugs has been found in the traditional medicines practices and in view of this it is important to undertake studies pertaining to screening of the folklore medicinal plants for their proclaimed anthelmintic efficacy **(Ahirrao *et al*., 2011).**

In the Study of **[Yadav *et al.,* 2006).** on various antifungal properties of essential oil of *Mentha spicata* L.var. MSS-5 showed cidal effect on mycelial growth of test fungi viz., 1100 ppm against *Aspergillus ochraceus* Wilhelm, 1000 ppm against *Penicillum digitatum* Sacc and *Pyricularia oryzae* Cavara and 700 ppm against *Alternaria alternata* .

In research of (Hajighasemi *et al.,* 2011). the cytotoxicity of aqueous extract of *M. spicata* on two tumor cell lines (Wehi-164 fibrosarcoma and U937 leukemic monocyte) has been evaluated *in vitro*, Aqueous extract of *M. spicata* significantly reduced the proliferation of Wehi-164 and U937 cells dose and time-dependently. The LD 50 values of *M. spicata* extract were 5.97, 4.63 and 4.77 mg/ml for the Wehi-164 cells and 5.6, 5.3 and 4.84 mg/ml for the U937 cells, after 24, 48 and 72 h treatment respectively. Aqueous extract of *M. spicata* showed cytotoxic effect in mouse fibrosarcoma Wehi-164 and human monocytic U937 cells. Thus, *M. spicata* could have potential anti-tumor activity.

Phytochemical screening of crude aqueous extracts of leaves of *Jatropha curcas*, *Vitex negundo*, *M. spicata* and flowers of *Delonix regia* revealed the presence of alkaloids, saponins, flavonoids and tannins, aqueous extracts of all the plants exhibited anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration, for *Pheritima posthuma* worms. The aqueous

extract of leaves of *M. spicata* caused paralysis is 10 min and time of death is 13 min while aqueous extracts of leaves of *Jatropha curcas*, *Vitex negundo*, and flowers of *Delonix regia* Rafin. revealed paralysis of 16, 17 and 12 min. and time of death 28, 37 and 18 min. respectively against *Pheritima posthuma*. The reference drug Piperazine citrate showed the paralysis at 22 min. and time of death at 100 mg conc. 49 min. respectively (Ahirrao *et al*., 2011).

References

Ahirrao R. A.1; Patel, M.R.; Hamid, S. ; Patil, J. K.; Suryawanshi, H.P. and Tadavi, S.A.1(2011). In vitro anthelmintic property of various herbal plants extracts against *Pheritima posthuma* . J. Pharmacologyonline 2: 542-547

Al-Hindi, A.I. and Lubbud, A.M.H. (2006). *Trichomonas vaginalis* infection among women: prevalence and trends during 2000-2006. Turk. J. Med. Sci.; 36(6): 371-375.

Al-Zabady,S.W.K.(2004).Isolation and Identification of *Trichomonas vaginalis* from trichomoniasis patients in Najaf city. M.Sc. Thesis. College of education, Kufa Univ.75 pp.(in Arabic).

Baser, K. (1993).Essential oils of Anatolian Labiatae: a profile. Acta Horticul.; 333: 217 -238.

Burstein,G.R. and Zenilman, J. M.(1999). Nongonococcal urethritis—a new paradigm. Clin. Infect. Dis. 28(Suppl. 1):S66–S73.

Hajighasemi, F. ; Hashemi, V. and Khoshzaban, F.(2011). Cytotoxic effect of *Mentha spicata* aqueous extract on cancerous cell lines *in vitro*. J. of Medicinal Plants Research Vol. 5(20), pp. 5142-5147,

Hooker, C.; Lott, W. and Harrich, D. (2001). Inhibitors of human immunodeficiency virus type 1 reverse transcriptase target distinct phases of early reverse transcription. J. Virol.;75:3095–3104.

Hussain, A.I.; Anwar, F.; Shahid, M.; Ashraf, M. and Przybylski, R. (2010). Chemical composition, antioxidant and antimicrobial activities of essential oil of spearmint (*mentha spicata L.*) from Pakistan. J. Essential Oil Research.;22:78-84.

Kengne, P.; Veas, F. ;Vidal, N. ;Rey, J. L. and Cuny. G. (1994). *Trichomonas vaginalis* repeated DNA target for highly sensitive and specific polymerase chain reaction diagnosis. Cell. Mol. Biol. (Noisy-Le-Grand) 40:819–831.

Kritikar, K.R. and Basu, B.D. (1975) .Indian Medicinal Plants, Bishen Sing Mahendra Pal Sing.Dehradun.2nd.;1511-1513.

Madico, G.; Quinn, T. C.; Rompalo, A.; Mckee, K. T.; Jr. and Gaydos, C. A. (1998). Diagnosis of *Trichomonas vaginalis* infection by PCR using vaginalswab samples. J. Clin. Microbiol. 36:3205–3210

Marquardt, W.C.; Demaree, R.S. and Grieve, R.B.(2003).Prasitology and Vector Biology. 2nd ed. Harcourt academic press.pp.73-87.

Mendoza-lo'pez, M.R. ; Becernil-Garcia, C. and Fattel-Facenda, L.V.(2000). Cp30, acysteine proteinase involved in *Trichomonas vaginalis* cytoadherence .Infect Immune ;68:4907-12.

Nadkarni, K.M. (2002). Indian materia medica. Ramdas Bhatkal for Popular Prakashan Pvt.Ltd. 3rd ed: Mumbai.;1186-1188.

Najaran, Z.T.; Firoozi,E.T.; Nasiri, R.; Jalali, N. and Hassanzadeh, M.K. (2013) Antiemetic activity of volatile oil from *Mentha spicata* and *Mentha piperita* in chemotherapy-induced nausea and vomiting. E cancer medical science J.;7:290.

Nasir, J.A.; Najam, J. ;Tahir, F. ;Asghar, M.N.and Iqbal, J.(2005).*Trichomonas vaginalis* in vaginal smears of women using Interauterine Contraceptive Device .Pak. J. Med. Res.; 44(3):144-116.

Petrin, D.; Dalgaty, K.; Bhatt, R. and Garber. G. (1998). Clinical and microbiological aspects of *Trichomonas vaginalis*. Clin. Microbiol. Rev. 11:300–317.

Riley, D. E.; Roberts, M.C.; Takayama, T.and Krieger J.N.(1992). Development of a polymerase chain reaction-based diagnosis of *Trichomonas vaginalis*. J. Clin. Microbiol. 30:465–472.

Ryu, J.S. ; Lee, M.H. ;Park, H. and Kang, J.H. (2002).Min, Dy.Survival of *Trichomonas vaginalis* exposed on various invironmental conditions. Infect Chemather ;33:373-379 .

Sanbongi, C.; Takano, H.; Osakabe, N.; Sasa, N.; Natsume, M.; Yanagisawa, R.; Inoue, K. ;Kato, Y.; Osawa, T.; and Yoshikawa, T.(2003). Rosmarinic acid inhibits lung injury induced by diesel exhaust particles. Free Radic Biol Med.;34*:*1060–1070

Satyavati, G.V. (1990). Use of Plants Drugs in Indian Traditional System of medicines and their relevance to Primary Health Car, In: N. R. Farnsworth, H. Wagner, eds. Economic and Medicinal Plant Research, Vol. IV, London: Academic Press Ltd;. p.190.

Schwebke, J.R. and Burgess, D. (2004). Trichomoniasis. Clin Microbiol Rev; 17:794-803,table of contents.

Schwebke, J.R. and Hook, E.W. (2003). High rates of *Trichomonas vaginalis* among men attending asexually transmitted diseases clinic:Implication for screening urethritis management .J. Infect. Dis.;188(3):465-8.

Shaio, M.F.; Lin,P.R. and Liu, J.Y.(1997). Colorimetric one-tube nested PCR for detection of *Trichomonas*

vaginalis in vaginal discharge. J. Clin Microbiol. 35:132–138.
Shehabi, A.A.; Awwad, Z.m.; AL-Ramahi, M.; Charvalos, E. and Abu-Qatouseh, L.F.(2009). Detection of *Mycoplasma genitalium* & *Trichomonas vaginlis* infections in General Jordanian Patients.American J.of Infections Disease;5(1):7-10.
Shetty, K. (2001). Biosynthesis and medical applications of rosmarinic acid. J. Herbs Spices Med. Plants.;8*:*161–183.
Smith,S.D.(2008). Trichomoniasis. Medline Article (WWW.) medicine from well, M.D.
Starburck, J. (2001).Herbs for sleep and relaxation. Men's Health.;16:24–26.
Sutherest, R.W.(2001). The valor ability of animal and human health to parasite under global change. Int.J.Parasitol., 31:933-948.
Thomson, D.P and Geary, T.G. (1995). The structure and function of helminthes surfaces. In: J. J. Marr, eds. Biochemistry and molecular biology of parasites, 1st ed. New York: Academic Press;. p. 203-32.
Uneke,C.J.;Alo, M.N. ; Ogbu,O. and Vgwuorn, D.C.(2007). *Trichomonas vaginalis* infection in human immunodeficiency virus – seropositive Nigerian women: the public health significance .J. Health. Mid. Sci.;6(2):1-7.
Weise, W. and Patel, S.C. (2000). Ameta-analysis of papanicolaou. smear and wet mount for diagnosis of vaginal trichomoniasis. Am. J. Med.108;101-108.
Yadav, R. S. ; Kumar S. and Dikshit, A. (2006). Antifungal properties of essential oil of *Mentha spicata* L.var. MSS-5. Indian J. Crop Science, 1(1-2): 197-200.
Yousuf, P. M. H.; Noba, N. Y. ; Shohel, M.; Bhattacherjee, R. and Das, B. K.(2013). Analgesic, Anti-Inflammatory and AntipyreticEffect of *Mentha spicata* (Spearmint). British J. of Pharmaceutical Research, 3(4): 854-864.