

Study on prevalence of *Trichomonas vaginalis* in Samarra city with observation of alcoholic extract effect of *Peganum harmala* plant *in vitro*

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Abstract

Background: Although metronidazole (MTZ) is widely used to treat trichomoniasis, the prevalence of metronidazole-resistance and its unpleasant adverse effects are well recognized. This drew the attention to the investigation of other lines of treatment, as that of herbal medicine. *Peganum harmala* plant proved to have important medicinal role. Some studies proved the antibacterial, antiprotozoal and antihelminthic properties of *P. harmala* plant. **Objective:** The present study aimed to investigate the effects of crude alcoholic extract of *P. harmala* on *Trichomonas vaginalis* growth *in vitro* and motility in comparison to MTZ. **Methodology:** Alcoholic extract were prepared from seeds of *P. harmala* plant. Different concentrations of *P. harmala* plant and MTZ were tested for their effect on the growth and motility of *T. vaginalis* trophozoites maintained in Diamond's TYM medium. **Results:** Present results showed the percentage of total infection was 62% with 100% sensitivity according to mode of direct wet mount and culture media by Diamond's TYM medium. Infection ratio varied according to age groups, where highest percentage of infection was 74% at 21-25 years old, followed 70% at 31-35 years old, while lowest percentage was 47% at 15-20 years old. Present results showed infection ratio in non-pregnant and pregnant women 62% and 0% respectively, as for the symptoms, the percentage of vaginal secretion reach to 70%, while percentage of itchy feeling was least (60%). Parasite numbers began with replication and increased during a period 24-48 hours from growth then began to decrease during a period 72-96 hours, so that 72 hours from growth considered logarithmic phase of *T. vaginalis*. Existing results evident to toxic effect of *P. harmala* at concentrations (150, 250, 350 and 450) mg/ml on *T. vaginalis* by observing gradual decrease of parasite numbers with concentrate increase of extract an inverse relationship during growth periods (24, 48, 72 and 96) hours. **Conclusions:** *P. harmala* had a statistically highly significant inhibitory effect on *T. vaginalis* as that of MTZ. This suggests that *P. harmala* may be promising phytotherapeutic agent for trichomoniasis.

Key words: *T. vaginalis*, *P. harmala*, *In vitro*.

دراسة وبائية لطيفي المشعرات المهبلية *Trichomonas vaginalis* في مدينة سامراء مع ملاحظة تأثير المستخلص الكحولي لنبات الحرمل *Peganum harmala* في الزجاج

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الخلاصة

الخلفية: يستخدم الميترونيدازول على نطاق واسع لعلاج داء المشعرات المهبلية، الا ان هناك الكثير من المخمجات اصبحت مقاومة للميترونيدازول ولوحظ ان للعقار آثار سلبية غير مرغوب بها. وقد ادى هذا إلى اللجوء الى استخدام

بدائل اخرى في العلاج، مثل العلاج بالأعشاب. أثبتت بعض الدراسات ان للحرمل خصائص مضادة للجراثيم، الطفيليات الابتدائية والديدان الطفيلية. **الهدف:** هدفت الدراسة الحالية إلى دراسة تأثير المستخلص الكحولي الخام لبذور نبات الحرمل على نمو المشعرات المهبلية مقارنة مع الميترونيدازول في الزجاج. **طرائق العمل:** حضر المستخلص الكحولي من بذور نبات الحرمل، واختبر تأثير تراكيز مختلفة منه ومن الميترونيدازول على نمو طفيلي المشعرات المهبلية في وسط دايمودن TYM. **النتائج:** أظهرت النتائج الحالية ان النسبة المئوية للإصابة الكلية كانت 62% مع حساسية 100% لكل من طريقة المسحة المباشرة الرطبة والزرع السائل في وسط دايمودن. لوحظ وجود اختلاف في نسبة الإصابة تبعاً للفئات العمرية، حيث بلغت أعلى نسبة إصابة 74% في الفئة العمرية 21-25 سنة، ثم 70% في الفئة العمرية 31-35 سنة، في حين كانت النسبة الأدنى 47% في الفئة العمرية 15-20 سنة. أظهرت النتائج الحالية ان نسبة الإصابة لدى النساء غير الحوامل والنساء الحوامل، 62% و 0% على التوالي، أما بالنسبة للأعراض، فإن نسبة إفرزات المهبل وصلت إلى 70%، في حين أن نسبة الشعور بالحكة كانت 60%. بدأت أعداد الطفيليات بالتضاعف خلال فترة 24-48 ساعة من النمو ثم بدأ العدد بالانخفاض خلال فترة 72-96 ساعة، حيث عدت الفترة 48 ساعة من النمو مرحلة الطور اللوغاريتمي لطفيلي المشعرات المهبلية. أظهرت النتائج الحالية التأثير السمي لنبات الحرمل عند التراكيز (150، 250، 350 و 450) ملغم/مل على طفيلي المشعرات المهبلية من خلال ملاحظة الانخفاض التدريجي في أعداد الطفيليات مع زيادة تركيز المستخلص اي بعلاقة عكسية خلال فترات النمو (24، 48، 72 و 96) ساعة. **الاستنتاجات:** لوحظ ان لنبات الحرمل تأثيراً مثبتاً عالياً لنمو طفيلي المشعرات المهبلية كما هو الحال للميترونيدازول. لذا يمكن ان يعد نبات الحرمل علاجاً جيداً كمضاد لداء المشعرات المهبلية.

كلمات المفتاح: طفيلي المشعرات المهبلية، نبات الحرمل، في الزجاج.

Introduction

T. vaginalis is a cosmopolitan, find in the reproductive tract of both men and women, it lives in the vagina and urethra of women, prostate and seminal vesicles of men, and it is transmitted primarily by sexual intercourse (Roberts and Janovy, 2009). Trichomoniasis is worldwide importance especially in recent years (Lazenby *et al.*, 2013), it has included a range of symptoms and it also has long-term effects, especially in women and it is associated with a variety of serious complications including preterm labour, low birth weight of newborns, cervical cancer and implicated in amplifying human immunodeficiency virus (HIV) transmission (Kissinger *et al.*, 2009; Hillier, 2013; Kissinger & Adamski, 2013). Muzny *et al.* (2013) referred to *T. vaginalis* is widespread among infected women with HIV, where observation of infection ratio with *T. vaginalis* in infected women with HIV was 17.4% comparison to other sexually transmitted diseases (STDs) that were less prevalent among these women, and he referred to *T. vaginalis* cause the infection of 250 million new cases annually in throughout

world (Parent *et al.*, 2013). *P. harmala* is a colonist succulence herb, grow to long 0.5m-1.5m, it leafs non-regular branched consist from 3-5 shiny green parts and 2-3cm tall. The stem is semi winding and branched. The flowers are white color (Kartesz, 2000). May be biological and medical efficacy of *P. harmala* belong to seeds contains (alkaloids) which are important such as (Harmine, Harmiline, Harmol and Harman), and other contains such as Resins (Budavari and Neil, 1996). *P. harmala* have used in India for syphilis treatment, for fever, hysteria and malaria treatment in north Africa (Boulus, 1983).

Aims of present study

Study of *T. vaginalis* prevalence among married women in Samarra city by using many diagnostic methods and exposure trying of *P. harmala* efficacy for the parasite inhibition in vitro.

Materials and Methods

100 samples were collected between August /2016 – January /2017 and investigation of

the samples taken by speculum and cotton swab by Gynecologist then added to each sample 2ml normal saline then transport immediately to laboratory for direct smear, the parasite has been diagnosed by its jerky movement, flagella motile and undulating membrane among epithelial cells (WHO, 1991). The stains below mentioned used in microscope examination for diagnosis sureness. Questionnaire sheet information included the name, age, pregnancy, associated symptoms and type of drug usage during a week of sample taken.

Method of staining

T. vaginalis stained according to Manson–Bahar and Bell (1987)

1-Thin smear made by putting one drop from positive culture media on slide and it distribute then let for dry.

2-The smear fixed by putting some drops ethanol 95% for ten minutes then it let for dry to next day.

3-The stain diluted by PBS solution, by taking 1ml of stain and 19ml of PBS then placed in staining container and the glass slide flooded for an hour.

4- The slide washed with tap water (by slow).

5-The slide let for dry then examined by the microscope at X100.

Preparation alcoholic extract of *P. harmala*

It prepared according to Harborne (1984).

1-Test for alkaloid

Dragendorff's test: To 1ml of the test solution, 1ml of Dragendorff's reagent was added. Formation of orange or orange red precipitate indicates to the presence of alkaloids (Mahmoud, 2008).

2-Tests for flavonoids

Ferric chloride test: 1ml of the test solution mixed with few drops of neutral ferric chloride solution, formation of blackish red colour indicates the presence of flavonoids (Rashant *et al.*, 2012).

3-Tests for glycosides

Sodium hydroxide test: The extract solution was mixed with equal amount of aqueous solution of 5% sodium hydroxide, formation of a yellow colour indicates the presence of glycosides (Harborne, 1984).

4-Test for phenolic compound and tannin

Ferric chloride test: To 1ml of extract, a few drops of 0.1% ferric chloride solution was added. Formation dark blue or greenish black colour solution indicates the presence of tannins or phenolic compounds, while brown colour indicates the presence of pseudo tannins (Kokate *et al.*, 2009).

5-Test for resins

To 1ml of extract, 15ml of 0.96% ethanol, then put the mixture in beaker contain 20ml of D.W. formation of precipitate indicates the presence of resins (Obidoa *et al.*, 2009).

Mode of culture for detection of *T. vaginalis* in vitro

The samples cultivated (which revealed negative and positive result by direct wet mount) by Diamond's TYM medium

$$n = \frac{(\text{Log N Log No})}{\text{Log 2}} = \frac{(\text{Log N Log No})}{0.301}$$

(Diamond, 1957).

The number of parasites count

The number of generations and generation time counted by the following two laws (Benjamin & German, 1993):

$$\text{Percentage of inhibition} = \frac{\text{Mean of parasite numbers of treated group} - \text{Mean of parasite numbers of control group}}{\text{Mean of parasite numbers of control group}} \times 100$$

Include:

n= Number of generation

N= Number of cells at time (t)

No= Number of start cells 2.29×10^5 cell/ml.

$$g = \frac{t}{n}$$

Include:

g= Generation time (hours)

t= Incubation period

n= Number of generation.

Testing the effect of Metronidazole and *P. harmala* extract on *T. vaginalis* trophozoite in vitro

Metronidazole (50, 100, 150 and 200) $\mu\text{g/ml}$ and *P. harmala* extract (350, 450, 550 and 650) mg/ml was used to study the effect of each drug and *P. harmala* extract on *T. vaginalis* trophozoite. Percentage of growth and inhibition detected and inhibitory concentration₅₀ (IC₅₀) detected too through logarithmic phase according to the following two laws:

Percentage of growth= 100 - Percentage of inhibition

Statistical analysis

Statistical program (Minitab) was used for analyzed data by using Duncan's multiple range test, analysis were performed probability values less than 0.05 were considered statistically significant (Elsahookie, 1990).

Results and Discussion**Percentage of total infection with *T. vaginalis***

Present results revealed total ratio of infection were 62% from total number of samples, note table (1).

Table (1): The percentage of total infection with *T. vaginalis* in Samarra city

Present study samples				
Total number	Positive samples No.	(Infection) (%)	Negative samples No.	(Non-infection) (%)
100	62	62	38	38

Present results did not agree with Al-badry (2013) and Almbashaa (2014) results in Samarra city, where the total ratio was 14.76% and 2.34% respectively. This variance in rates belong to different in number and type of samples, where some studies reported highest ratio of infection was during vaginal secretion examination comparison to urine examination, due to urinary canal and reproductive canal in women was separated, so the vagina is normal place for *T. vaginalis* but when the parasite proliferate and due to crowded and competition on nourishment and available space, it will penetrate urinary canal, so that it appearance in urine considered incidental event (Al-Ebrahimi, 2008). The sexual life manner consider one of factors which cause increase of trichomoniasis diffusion (Verteramo *et al.*, 2008), and variance in rates return to time different in samples

examination, so should the samples examine during one hour from samples taken, the disparity in rates may be due to the time difference of the samples examination, Stoner *et al.*, (2013) referred to necessity of samples examined within one hour of collection it, has been noted that the parasite movement was 100% in the first thirty minutes of taking the sample and 99% after 60 minutes of taking the sample and decreased the ratio 3-15% every hour later.

Diagnostic study

Present results showed a contrast infection ratio, depending on the method used in the diagnosis, the highest ratio of infection was 62% and sensitivity of up to 100% by using direct wet mount and the method of liquid cultivate by Diamond's TYM medium. Table (2).

Table (2):- the percentage of infection depending on methods of diagnosis and it sensitivity

Modes of diagnos	The total No.	Positive samples No.	(Infection) (%)	Negative sample No.	(Non-infection) (%)
Direct examine	100	62(a)	62	38(b)	38
Culture media		62(a)	62	38(b)	38

a=positive correct reading, b= negative reading

$$\text{Sensitivity\%} = \frac{a}{a+b} \times 100$$

$$\text{direct wet mount} = \frac{62}{62+38} \times 100 = 62\%$$

$$\text{liquid cultivate} = \frac{62}{62+38} \times 100 = 62\%$$

The present study recorded a high sensitivity (62%) of direct wet mount and liquid cultivate methods, Al-Ebrahimi (2008) indicated to the sensitivity rate of direct wet mount and liquid cultivate, were 88.4% and 82.6%, respectively, and Mushref *et al.* (2011) indicated to the sensitivity rate of direct wet mount 66.7% and liquid cultivate 100% by using CPLM medium. Patil *et al.* (2012) revealed the sensitivity rate of direct wet mount and liquid cultivate, were 60% and 73.33%, respectively, this variation in efficiency of methods used in the detection of parasite belong to used stains in direct wet mount and medium type different used in diagnosis. Direct wet mount sensitivity may be less compared to the liquid cultivate, due to the parasite numbers lack in the samples examined by direct wet mount manner and

accompanied with other infections (McCann, 1974) or due to the parasite loss of distinct movement and depended it in diagnosis, due to the different of it environment (Petrin *et al.*, 1998; Shehbi *et al.*, 2009), and direct wet mount sensitivity still related to the improvements in examination manner, such as an examination in local of smear taken (Patil *et al.*, 2012).

Pregnancy

Not-pregnant women have formed percentage (62%) of infection with trichomoniasis, while didn't reported infection rate for pregnant women because not investigated any pregnant women sample due to not come any pregnant women complain of clinical symptoms (table 3).

Table (3):- The percentage of infection with *T. vaginalis* according to pregnancy

Pregnancy	The investigate No.	Infected No.	Percentage (%)
Pregnant	0	0	0
Non-pregnant	100	62	62
Total	100	62	62

The present results agreed with the results of Al-Ebrahimi (2008) and Dahab *et al.* (2012), where they reported the highest rate of infection in non-pregnant women 5.6% and 13.3%, respectively, as the present results agreed with reported of AL-Badry

(2013), where she indicated to the non-pregnant women formed the highest percentage of infection (90.32%), pregnant women followed by (9.68%). This result gives further evidence of the importance of clinical examination for early diagnosis and

treatment of infection in pregnant women, also advised them for regular visit to healthy centers for pregnancy check (Adeoye & Akande, 2007). The present results did not agree with reported of each Al-Samarraie (2002) and Muzher (2008), where they reported highest percentage of

infection in pregnant women were 13.06% and 28.5% respectively.

Age groups

The present results showed that the highest rate of vaginal infection 74% in the age group 21-25 years, followed by 70% in the age group 31-35 years, while the lowest percentage was 47% in the age group 15-20 years (table 4).

Table (4):- the percentage of infection with *T. vaginalis* according to age groups

Age groups	The investigat No.	Infected No.	Percentage (%)	Non-infected No.	Percentage (%)
15-20	15	7	47	8	53
21-25	19	14	74	5	26
26-30	17	10	59	7	41
31-35	23	16	70	7	30
36-40	14	7	50	7	50
>40	12	8	67	4	33
Total	100	62	62	38	38

The present results did not agree with results of Al-Ebrahimi (2008) and Hussain (2010), where the highest rate reached (7.6% and 31.50% in Basra and Babel respectively) for trichomoniasis in the age group (26-30 years. the present results did not agree with results of Khalil *et al.* (2012) in Baghdad, where the infection ratio reached to 27.8% in the age groups (25-29 years) and (30-34) years. As well as the present results do not agree with results of AL-Badry (2013), where her indicated to the highest rate of infection in women was (29.03%) in the age group (26-30 years), followed by 22.58% in the age group (31-35 years), as the present results do not agree with results of Almbashaa (2014), which

showed that the highest rate of infection reached to 4.16% in the age groups (30-40 years) followed by the age group 29-19 years by 1%. The infection causes in the age group (21-25 years) belong to it were years of marriage addition to of the sexual hormones access highest level (Jawetz *et al.*, 1998), where studies have indicated to reproductive hormone (estrogen) levels may be partly responsible for prevalence increase of trichomoniasis, this hormone is reduced in older women and it is increasing in young women (Buv'e *et al.*, 2001 ; Mahdi *et al.*, 2001). as the loss or low of infection with age growing attribute to sexual inactivity, which represented by decrease of estrogen hormone and pH of the

vagina (Zhang *et al.*, 1995). Secretion of estrogen hormone stops after menopause (where there is a gradual atrophy of the genitals, and size at least of the uterus and the vagina wall becomes thin and smooth with a decrease in the secretion of acidity and the vagina environment more alkaline becomes and this medium can settle on the parasite's growth (Arroyo *et al.*, 2006; Stark *et al.*, 2009). The estrogen hormone causes increase vaginal secretions and acidic makes it by glycogen lysis and convert it into lactic acid (Marquardt *et al.*, 2003). The differences in the studies results

attribute to a few reasons such as education level, personal hygiene and living situation and the women involved in the study (patients or not) (Spencer, 1990).

Clinical symptoms

All of infection women complain of different clinical symptoms such as vaginal secretions represented 70%, lower abdominal pain 63%, smelly 90% and burning sensation 70%, while women who complain of itching represented of lowest rate 60% (table 5).

Table (5):- The percentage of infection with *T. vaginalis* and clinical symptoms associated with them

Clinical symptoms	Total number of samples	Infected No.	Percentage (%)	Non-infected No.
Vaginal secretions	57	40	70	17
Lower abdominal pain	19	12	63	7
Smelly	11	10	90	1
Itching	66	40	60	26
Burning sensation	64	45	70	19

Results of this study agree with many of the studies which indicated to the infection with *T. vaginalis* associated with these symptoms (table 5), especially vaginal secretions (Al-Hindi & Lubbed, 2006; Adeoye & Akande, 2007), the present results do not agree with a results of each Al-Ebrahimi (2008), Dahab *et al.* (2012) and Patil *et al.* (2012), where they reported the highest percentage of vaginal secretion was 58.4%, 15.5% and 18% respectively, and the most prominent of clinical symptoms of infection presence of copious discharge, as well as these secretions be

denser than usual or watery (Breeding, 1996). also through the present results noted presence of other clinical symptoms including lower abdominal pain. When the infection effect on vaginal epithelial layer, the wall will fully influenced and the pain increase when pressure on the lower abdomen (AL-Zubaidi, 2005). Sulyman (2008) indicated to the existence of a clear relationship between *T. vaginalis* and itching in women who suffer from vaginal discharge, where the rate of infection reached to 4.8%, while the lowest percentage was 1.4% with vaginal itching

absence, and the reason attributed to the appearance of irritation and itching symptoms to rapid rotational movement of

the parasite, flagella motile and caudal tip tapered (Hayes *et al.*, 2002).

Chemical detection of some active compounds in alcoholic extract of *P. harmala*

Present results showed alcoholic extract of *P. harmala* contain on some active

compounds (table 6), include alkaloids, flavonoids, glycosides, phenols, resins and tannins.

Table (6):- active compounds in Alcoholic extract

No.	Type of test	Alcoholic extract
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Phenols	+
5	Resins	+
6	Tannins	+

The present results agreed with results of AL-juboori (2009), about *P. harmala* contain on alkaloids, glycosides, resins and tannins. Also they agreed with results of Wazzan (2009), about *P. harmala* contain on alkaloids, flavonoids, glycosides and resins.

The effect of Metronidazole drug on *T. vaginalis* in vitro

Different concentrations Used of the drug as chemical therapy and control model, the results of the drug effect on the parasite the

follow-up daily, where present results shown (after 24 hours of concentration added) a high reduction in number and activity of the parasites at all concentrations of the drug used, especially 150 and 200 µg/ml, the parasites disappeared completely after 96 hours (table 7), also the inhibitory concentration₅₀ (IC₅₀) of the parasites was 100 µg/ml (after 48 hours of concentration added).

Table (7):- The effect of different concentrations of metronidazole on *T. vaginalis* in limited periods of growth (average of parasites number $\times 10^5$ /ml).

Incubation Period (hour) Treated groups ($\mu\text{g/ml}$)	24			48			72			96		
	Mean \pm SD	Growth (%)	Inhibition (%)	Mean \pm SD	Growth (%)	Inhibition (%)	Mean \pm SD	Growth (%)	Inhibition (%)	Mean \pm SD	Growth (%)	Inhibition (%)
Control	4.27 \pm 0.81 A	100	-----	7.02 \pm 0.96 A	100	-----	5.88 \pm 1.01 A	100	-----	4.08 \pm 0.86 A	100	-----
50	4.28 \pm 0.70 A	99.77	0.23	2.83 \pm 0.66 B	40.32	59.68	1.02 \pm 0.53 B	17.35	82.65	0.00 \pm 0.00 B	0.00	100
100	5.04 \pm 1.35 A	81.97	18.03	2.87 \pm 0.66 B	40.89	59.11	0.65 \pm 0.23 B	11.06	88.94	0.00 \pm 0.00 B	0.00	100
150	4.57 \pm 1.37 A	92.98	7.02	2.27 \pm 0.61 B	32.34	67.66	0.20 \pm 0.11 C	3.4	96.60	0.00 \pm 0.00 B	0.00	100
200	3.98 \pm 1.04 A	93.21	6.79	0.64 \pm 0.26 C	9.12	90.88	0.00 \pm 0.00 D	0.00	100	0.00 \pm 0.00 B	0.00	100

Similar letters indicate to non-significant differences ($P > 0.05$) among groups (vertical compare). Different letters indicate to significant differences ($P \leq 0.05$) among groups (vertical compare). Table (7) revealed significant statistical differences between the of some groups treated on the one hand and between them and the control group on the other hand, especially after 72 hours of added concentrations under study. Metronidazole belongs to the 5-nitroimidazoles group and it considers first choice for trichomoniasis treatment. (Harris *et al.*, 2001). This drug is administered orally or intravenously. Bioavailability of 93-100% because metronidazole does not bind to serum proteins and enter the cell and its organelles via facilitated diffusion (Houghton *et al.*, 1979 and Harris *et al.*, 2001), then the drug activated by the reduced of hydrogenosomal ferredoxin via reduction of the 5-nitro group (Lindmark, 1980 and Muller, 1986). The efficiency of this drug is high, with a cure rate of 85-95% of treated patients and re-infections can be

avoided through simultaneous treatment of sexual partners (Lossick, 1990). Chemotherapy commonly using metronidazole causes mild side effects, characterized by the body's defence mechanisms against toxic substances, like nausea, vomiting, diarrhea, dizziness and headache. More serious side effects, like anorexia, hypersensitivity, leukopenia, palpitation, confusion, encephalopathy, and peripheral neuropathy are clinically rarely observed (Lossick, 1990). The present results do not agree with reported of Ahmed (2010), where he indicated to inhibition a ratio 100% of *T. vaginalis* in vitro (after 48 hours of MTZ concentrations added) at concentrations of 50 and 100 $\mu\text{g/ml}$. As the present results do not agree with the results of Sulyman (2008), where he stated the percentage of inhibition of MTZ was 100% for some concentrations (1.25%, 2.5%, 0.5% and 10%) used after 48 hours of added and the percentage of inhibition was 100% for some other (0.5 % and 0.75%) after 72 hours of added, he has pointed out

to the parasite has resistance to MTZ concentrations of low-lying.

The effect of alcoholic extract of *P. harmala* on *T. vaginalis* in vitro

The table (8) shows alcoholic extract effect of *P. harmala* plant on the number and activity of *T. vaginalis* in vitro, where the

concentrations (150 and 250) mg/ml were approach in the effect on *T. vaginalis* growth, while concentrations (350 and 450) mg/ml were the best in the inhibitory effectiveness for the parasite growth after 72 hours of added. The inhibitory concentration₅₀ (IC₅₀) of *T. vaginalis* was 250 mg/ml (after 48 hours of added).

Table (8):- The effect of different concentrations of alcoholic extract of *P. harmala* on *T. vaginalis* in limited periods of growth (average of parasites number $\times 10^5$ /ml).

Incubation Period (hour) Treated groups (μ g/ml)	24			48			72			96		
	Mean \pm SD	Growth (%)	Inhibition (%)	Mean \pm SD	Growth (%)	Inhibition (%)	Mean \pm SD	Growth (%)	Inhibition (%)	Mean \pm SD	Growth (%)	Inhibition (%)
Control	4.27 \pm 0.81 B	100	-----	7.02 \pm 0.96 A	100	-----	5.88 \pm 1.01 A	100	-----	4.08 \pm 0.86 A	100	-----
150	5.67 \pm 0.58 A	67.21	32.79	4.06 \pm 0.56 B	57.83	42.17	1.82 \pm 0.34 B	30.96	69.04	0.00 \pm 0.00 B	0.00	100
250	5.17 \pm 0.52 A	78.92	21.08	2.90 \pm 0.10 C	41.31	58.69	0.54 \pm 0.24 B	9.19	90.81	0.00 \pm 0.00 B	0.00	100
350	4.24 \pm 0.42 B	99.29	0.70	1.12 \pm 0.38 D	15.96	84.04	0.00 \pm 0.00 C	0.00	100	0.00 \pm 0.00 B	0.00	100
450	3.91 \pm 0.32 B	91.57	8.43	0.35 \pm 0.14 E	4.99	95.01	0.00 \pm 0.00 C	0.00	100	0.00 \pm 0.00 B	0.00	100

Similar letters indicate to non-significant differences ($P > 0.05$) among groups (vertical compare).

Different letters indicate to significant differences ($P \leq 0.05$) among groups (vertical compare).

Through a table (8) observed non-significant statistical differences between treated groups and control group, with different concentrations (under study). Inhibitory effect of the extract may be due to it contains alkaloids (Hoffmann, 1996) or Albuliatrin (tannin component) which settle on a wide range of parasites (Moneam *et al.*, 1988). Or due to it contains the flavonoids (Artimeva, 1981). The cause may be belongs to the extract contains

phenols and tannins, or the presence of toxic substances in different proportions include the active substances or phenolic compounds cause may of toxic hydrogen peroxide production which has effective impact on parasite (Al-Tae, 2000; Wang, 1993 and Sulyman, 2008). Al-Juwayr (2006) indicated to glycosides and alkaloids have an important role in the inhibition of *T. vaginalis* growth. The alkaloids have an effective impact on *T. vaginalis* (Tagboto *et*

al., 2001). Berberine (alkaloid component) was used at 1mg/ml concentration to test its effect on *T. vaginalis* in vitro, where it causes vital loss of parasite by 34.2% after 72 hours of incubation, alkaloids (Berberine especially) intervention to the cell and linked with mitochondria DNA and inhibits of Topoisomerase II enzyme (Soffar *et al.*, 2001).

Determination of logarithmic phase

T. vaginalis numbers counted by using counting slide of blood cells (hemocytometer) after every 24 hours of

growth for four days, thus the log. phase identified of the parasite developing in Diamond's TYM medium (Figure 1). *T. vaginalis* numbers began multiply and increase during the period 24-48 hours of growth and then it began decrease during the period 72-96 hours, so that the period 72 hours considered the log. phase of the parasite growth. Table (9) revealed the generations number for *T. vaginalis* in a log. phase after 48 hours pass was 53.41 ± 7.28 , also it revealed the generation time after 48 hours was 0.92 ± 0.15 .

Table (9):- Average of parasites number ($\times 10^5$), average of generations number and average of generation time according to period of growth \pm SD.

The period (hour)	Number of parasites $\times 10^5$ /ml Mean \pm SD	Number of generations Mean \pm SD	Generation time Mean \pm SD
	4.27 \pm 0.93	32.50 \pm 6.16	0.76 \pm 0.17
	7.04 \pm 1.11	53.41 \pm 7.28	0.92 \pm 0.15
	5.88 \pm 1.17	44.73 \pm 7.70	1.66 \pm 0.33
	4.08 \pm 0.99	31.06 \pm 6.53	3.23 \pm 0.78

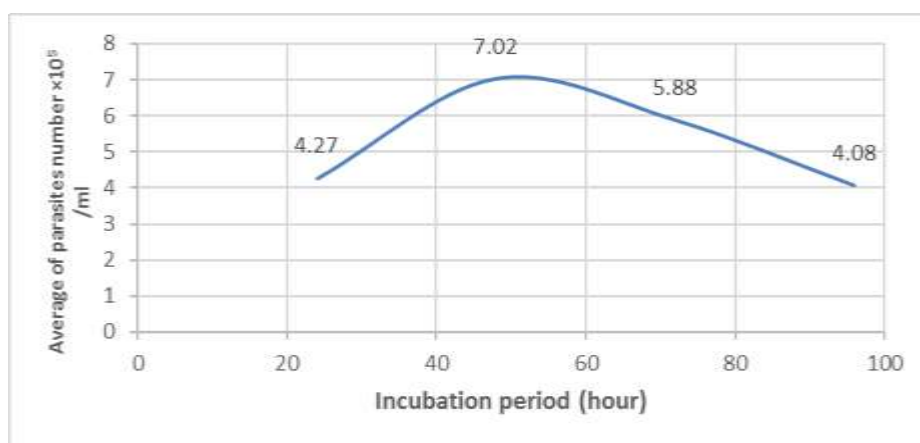


Figure (1):- Normal curve of *T. vaginalis* growth in Diamond's TYM medium

This result is an approach to reported of Belding (1965), where he referred to the log. phase of *T. vaginalis* limited between 24-96 hours of growth in vitro, the present results agreed with results of Castro-Garza *et al.* (1996), who confirmed that the log. phase of *T. vaginalis* developing up to 72 hours, either Kostara *et al.* (1998) pointed out that the log. phase does not exceed 144 hours, and the period determination of the log. phase depends on several factors, including growth medium acidic, primary number of cultivate and temperature.

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