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EFFECT OF AQUEOUS EXTRACT OF BLACK SEED(*NIGELLA SATIVA*) AND AQUEOUS EXTRACT OF *TRIGONELLAFOENUM* IN SOMEHISTOLOGICAL AND BIOCHEMICAL CHANGES TO*TRICHOMONASVAGINALIS*

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ABSTRACT

Trichomonasvaginalis is a unicellular protozoan flagellate parasite, that has only the trophozoites stage. It has no cystic stage in its life cycle. This study was designed to investigate the in vivo inhibitory activity of *Nigella sativa* and *Trigonellafoenum* aqueous extract on the growth of *Trichomonasvaginalis* in comparison to metronidazole, and determine effect of different concentrations of *Nigella sativa* and *Trigonellafoenum* seeds extract of pathological physiological, histological and Biochemical changes on mice infected with *Trichomonasvaginalis.* This study was carried out during the period from Noveber, 2015 to April, 2016. Vaginal swabs and urine samples were collected from AL-habebia hospital ad Ibn Al-balady hospital and Al-ulweiya hospitals for women and childbirth in Baghdad. Collect samples of women who suffer from the symptoms of the disease vaginitis, Itching and burning. swab specimen was immediately placed in 5mL of Diamond modified medium. 80 of female mice in age two month and weight (25-35 gm) given 10^5 trophozoites/ml in the vagina. During 6-day swabs taken from the vagina of mice and examined under light microscope in order to know the number of trophozoite and the impact of aqueous extract on the growth of parasites and compared with the control positive and with the treatment of metronidazole. The best results of the highest inhibition on the sixth day were for Flagyl, as well as treatment with *Nigella sativa* 10 mg/ml and the mixture of *N.sativa and T. foenum* 5 mg/ml followed from other transactions and significantly less than 0.01 compare with positive control. The Biochemical test of Glucose, Creatinin and Alkaline-phosphatase show Glucose level increase in Flagyl group (227.25 ± 8.9 b mg/dl) and its level increase in Control +ve (241.3 ± 1.3 a mg/dl), *N. sativa* 5 mg/ml

(193.54 ± 2.94 d mg/dl), *N. sativa* 10 mg/ml (211.66 ± 32.9 c mg/dl), *T. foenum* 5 mg/m (181.66 ± 24.6 d mg/dl), *T. foenum* 10 mg/ml (192.91 ± 1.4 d mg/dl), compared with Control –ve group (171.27 ± 0.9 e mg/dl). the difference was significant between all treatments except between the Control –ve and mixture *N.sativa* and *T.foenum* 5mg/ml(172.21 ± 27.2 e mg/dl) difference was non-significant (P<0.01). Level of creatinin in serum decrease in the rate in Flagyl group (0.14 ± 0.05 cmg/dl), *N. sativa* 5 mg/ml (0.145 ± 0.05 c mg/dl), *N. sativa* 10 mg/ml (0.135 ± 0.05 c mg/dl), *T. foenum* 5 mg/m (mg/dl), mixture *N.sativa* and *T. foenum* 5mg/ml (0.155 ± 0.09 c mg/dl) ,and Control + ve (0.155 ± 0.05 c mg/dl) compared with Control –ve group (171.27 ± 0.9 e mg/dl). the difference was significant between all treatments except between the Control –ve and *T. foenum* 10 mg/ml was non-significant (P<0.01). ALkaline-phosphatase level decrease in Flagyl group (119.82 ± 10.91 c U/l), *N. sativa* 5 mg/ml (125.77 ± 9.6 b U/l), *N. sativa* 10 mg/ml (125.19 ± 16.11 b U/l), *T.foenum* 5 mg/m (85.06 ± 1.71 d U/l), *T. foenum* 10 mg/ml (56.27 ± 2.5 e U/l), mixture *N.sativa* and *T.foenum* 5mg/ml (113.11 ± 17.9 c U/l), and in Control + ve (0.23 ± 0.08 U/l) compared with Control –ve group (186.67 ± 2.11 a U/l). the difference was significant between all treatments (P<0.01). Results showed diverse histopathological changes in the uterus and vagina of infected animals with *Trichomonasvaginalis* included inflammatory cellular infiltration, secretory activity and hyperplasia of epithelia cell.

KEYWORDS: *Trichomonasvaginalis Trigonellafoenum* histopathological cell.

1.1 INTRODUCTION

Trichomonasvaginalis is a unicellular protozoan flagellate parasite, that has only the trophozoites stage. It has no cystic stage in its life cycle. Infection with the organism, frequently asymptomatic, can cause inflammation of vagina in women and urethritis in men. Vaginal discharge is often yellowish-green in color, it may be associated with vulval itching, burning, and redness and swelling (Schmidt and Roberts, 2000). *Trichomonasvaginalis* is a human pathogen that causes the most common, non-viral Sexually Transmitted Diseases (STDs) in the world, infecting 248 million people yearly according to WHO estimates, with the majority living in developing countries (WHO, 2011).

Metronidazole has been so far the drug is useful to human Trichomoniasis. However, it can be used lead to drug resistance. Many it can beovercome with treatment-resistant cases for long periods and higher Dose of metronidazole, but sometimes patients continue to injury In spite of these measures (Blaha*et al*., 2006).

Nigella sativa (Family: Ranunculacea), commonly known as black seed, black cumin or habatul Barakah. It has been used for centuries as a spice, curative and food preservative or medicinal therapy for various ailments, including infectious diseases (Ali and Blunden, 2003). *Trigonellafoenum-graecum* seeds contains fatty oils, mucilage protein, mannogalactan, resin, trigonellin, cholin, saponin, tannin, essential oils, phosphorus, iron; contains no starch (Al-Rawi and Chakravarty, 1988). The aqueous and alcoholic extracts of *Trigonellafoenumgraecum* seeds have antileishmanial effect on Leishmania major parasites in vitro at different concentrations when compared to the control (Jarallah, 2005) and antibacterial (Bhatti, et. al., 1996) and antimicrobial (Alkofahi*et al*., 1996).

1.2 The aims of the present study

The aim of study is finding alternative treatment for Trichomoniasis instead of using Metronidazole and that stimulated to be the aim of the present study is.

1. A histological study to determine changes induced by *Trichomonasvaginalis*.
2. Study the efficiency of the seed extract of *Nigella Sativa* on parasite infection and comparison with Metronidazole.
3. Study the efficiency of the seed extract of *Trigonellafoenum*on parasite infection and comparison with Metronidazole.
4. Study the efficiency of the mix which consist from seed extract of *Nigella Sativa* and *Trigonellafoenum*on parasite infection and comparison with Metronidazole.
5. Biochemical study (glucose, enzymes) at different stages of the parasite infection.

2. MATERIALS AND METHODS

2.1Patients and samples

Trichomonasvaginalis samples collected from AL-habebia hospital and Ibn Al-balady hospital and Al-ulweiya hospitals for women and childbirth in Baghdad.Collect samples of women who suffer from the symptoms of the disease vaginitis,Itching and burning (Rein, 1990; Brown, 1975) Through the sterile vaginal speculum two samples of the vaginal discharge were taken by a doctor Women from the posterior fornix of the vagina by mean of sterile cotton swab one was immersed immediately into a sterile test tube containing I ml of sterile normal saline covered with sterile cotton plug this tube was kept warm as far as possible till the time of examination (Thomas *et al*., 1996). The second swab was put in a sterile empty test tube which was covered with sterile cotton plug as well.

2.2 Isolation of parasite

according to the method described by (Collee and *et al.*,1996).

Dye is to ensure detection of the presence of the parasite *Trichomonasvaginalis* coloring according to the method described by (Fouts and Kraus 1980). After placing cover slide to remark the movement of *Trichomonasvaginalis*,the counting of examination is positive in case of observe the shape and the movement of parasite (Thomason and Gelbart 1989) while counting the examination is negative when you are not see the parasite within period range from 3-5 minutes of examination continuous.

2.3 Cultural methods

The 'gold standard' for the diagnosis of trichomoniasis is broth culture technique using Diamond's medium according to the method described by (Diamond, 1957).

2.4Preparation of *Nigella Sativa*and *Trigonellafoenum*Aqueous Extract

2.4.1 *N. sativa* Aqueous Extract

Followed the way of (El Wakil, 2007) to get the extract, The present study evaluated two doses of NS AE: 5mg/ml and 10 mg/ml (Al-Heali and Rahemo 2006).

2.4.2*Trigonellafoenum*Aqueous Extract:

Followed the way of (Gruenwald et al., 1998) to get the extract**.** The present study evaluated two doses: 5 mg/ml and 10 mg/ml.

In addition to that it has been preparation a third extract by mixing doses (5 mg/ml) of *N. sativa* Aqueous Extract and *Trigonellafoenum* Aqueous Extract then preserved in the deep freezer (–20°C) till it was used.

2.5 Animals Laboratory

White female laboratory mice has been used from the strain (Balb/c) their ages ranged from(6-8)weeks, which has been obtained from the National Center for control and research of Drug. the mice has been placed in plastic cages (10 in each cage) covered with a metal cover and equipped with sawdust which was replaced by a week to maintain the cages cleaning, has also been taking observance the environmental condition such as the nutrition, aeration and temperature along the period of study.

2.6 Experimental Design

80 of female mice in age ages ranged from (6-8) weeks and weight (25-35 gm) given 10^5 trophozoites/ml in the vagina and after 48 hour all the mice vaginal were examined by used sterile cotton swab put on clean slide to be examined under the light microscope under the power 10X then 40X (Fouts and Kraus 1980) to make sure infected by parasite and after that the mice divided into groups each group contain 10 mice then inoculated as follows.

Group: 0.1ml from *N. sativa* Aqueous Extract in concentration 5 and 10 mg/ml.

Group: 0.1ml from *T. foenum*Aqueous Extract in concentration 5 and 10mg/ml.

Group: 0.1ml from *N. sativa* and *T. foenum* Aqueous Extract in concentration 5 mg/ml.

Group: 0.1ml from Flagyl.

Group: 0.1ml from normal saline and consider as positive control.

Group: leave without infection and consider as negative control.

During the 6 days a swab taken from the vagina, then put on slid and examined under light microscope to enumerate the number of parasite before and after inoculated with the *N. sativa*, *T. foenum* Aqueous Extract and Flagyl.

Evaluation of the drug efficacy was done by.

1. Counting the number of trophozoites using the haemocytometer (Neubauer cell-counter chamber).
2. Calculation of the percent of inhibition of multiplication according to the equation:
   Percent inhibition of growth = $\frac{a-b}{a} \times 100$ Where;

a=Mean number of trophozoites in control mice and

b= Mean number of trophozoites in treatment mice (Palmas *et al*., 1984)

2.7 Histological examination

The mice were killed and extracted the organs (uterus, vagina) and its carried by a string of successive processes, according to the method described by (Luna,1968)

2.8 Biochemical Test

Measurement of Creatinine, Glucose and Alkaline-phosphatase, They were measured in serum according to method of kit.

2.9 *Statistical Analysis*

according to (Shimano *et al*.,2004).

3. RESULTS AND DISCUSSION

3.1 Level of Glucose in the serum

Glucose level increase in Flagyl group (227.25 ± 8.9 b mg/dl), *N. sativa* 5 mg/ml (193.54 ± 2.94 d mg/dl), *N. sativa* 10 mg/ml (211.66 ± 32.9 c mg/dl), *T. foenum* 5 mg/m (181.66 ± 24.6 d mg/dl), *T. foenum* 10 mg/ml (192.91 ± 1.4 d mg/dl), mixture *N.sativa* and *T. foenum* 5mg/ml (172.21 ± 27.2 e mg/dl), and its level increase in Control +ve (241.3 ± 1.3 a mg/dl) compared with Control –ve group (171.27 ± 0.9 e mg/dl) . the difference was significant between all treatments except between the Control –ve and mixture *N.sativa* and *T.foenum* 5mg/ml difference was non-significant (P<0.01). As shown in the table.(3-1)

Table: (3-1) **compare between different group in glucose.**

Group	**Mean ± SD**
	Glucose mg/dl
Control –ve	171.27 ± 0.9 e
Control +ve	241.3 ± 1.3 a
Flagyl	227.25 ± 8.9 b
N. sativa 5 mg/ml	193.54 ± 2.94 d
N. sativa 10 mg/ml	211.66 ± 32.9 c
T. foenum 5 mg/ml	181.66 ± 24.6 d
T. foenum 10 mg/ml	192.91 ± 1.4 d
mixture *N.sativa and T.foenum* 5mg/ml	172.21 ± 27.2 e
LSD	11.4

Similar letters mean the absence of significant differences and different letters mean the presence of significant differences at the level of probability (P<0. 01).

The amount of glucose in the blood serum of infected mice and described in the table(3-1) shown increase where it was 241.3 ± 1.3 a mg/dl in infected mice compared with Control-ve group 171.27 ± 0.9 e mg/dl. *T. vaginalis*has the ability to adapt its energy metabolism extensively, as indicated by changes in both the rate of glucose transport across the plasma membrane and the specific activity of glucokinase (TerKuile, 1994). TerKuile and Müller (1994) have studied maltose operation and transport. In most eubacteria and yeasts, maltose is transported into the cell and then hydrolyzed to glucose (Schwartz, 1987). These investigators have demonstrated that as in the intestinal epithelial cells of vertebrates (Semenza, 1987), maltose is cleaved on the cell surface of the parasite to glucose via α-glucosidase. Glucose is subsequently transported by the glucose transporter into the cytosol, where it is metabolized. This process whereby the carbohydrate is metabolized on the membrane, does not seem to be advantageous for a unicellular organism, since glucose has

been found to diffuse away from the parasite under culture conditions (Ter Kuile and Müller, 1995), That explain the increase of glucose during infection with *T. vaginalis* .

The Control –ve and mixture *N.sativa* and *T.foenum* 5mg/ml difference was non-significant because Various reports have confirmed that *Trigonellafoenum-graecum* (fenugreek) seeds can lower blood glucose and cholesterol in type 1 and type 2 diabetics and experimental diabetic animals(Puri *et al*.,2002).and various reports have confirmed that *Nigella sativa* crude aqueous extract have effect to restore glucose homeostasis (Labhal*et al*., 1997), by enhancing glucose induced insulin release from beta cells in the islets of langerhans (Le *et al*., 2004).

3-2 Level of creatinin in serum

The result showed decrease in the rate of Creatine in Flagyl group (0.14 ± 0.05 cmg/dl), *N. sativa* 5 mg/ml (0.145 ± 0.05 c mg/dl), *N. sativa* 10 mg/ml (0.135 ± 0.05 c mg/dl), *T. foenum* 5 mg/m (mg/dl), mixture *N.sativa* and *T.foenum* 5mg/ml (0.155 ± 0.09 c mg/dl) ,and Control +ve(0.155 ± 0.05 c mg/dl) compared with Control –ve group (0.23 ± 0.08 mg/dl). the difference was significant between all treatments except between the Control –ve and *T. foenum* 10 mg/ml was non-significant (P<0.01). As shown in the table (3-2)

Table: (3-2) compare between different group in Creatinin.

Group	Mean ± SD Creatinin mg/dl
Control –ve	0.23 ± 0.08 a
Control +ve	0.155 ± 0.05 c
Flagyl	0.14 ± 0.05 c
N. sativa 5 mg/ml	0.145 ± 0.05 c
N. sativa 10 mg/ml	0.135 ± 0.05 c
T. foenum 5 mg/ml	0.17 ± 0.07 b
T. foenum 10 mg/ml	0.23 ± 0.07 a
mixture *N.sativa and T.foenum* 5mg/ml	0.155 ± 0.09 c
LSD	0.05

Similar letters mean the absence of significant differences and different letters mean the presence of significant differences at the level of probability (P<0. 01).

The amount of Creatinin in the blood serum of infected mice and described in the table (3-2) shown decrease in level compared with Control –ve group (171.27 ± 0.9 e mg/dl), Oterdoom et al.(2009) showed that low creatinine excretion, as an indirect measure of low muscle mass,

is associated with increased incidence of cardiovascular events and all-cause mortality, independent of insulin resistance.

Creatinin level decrease when the mass of muscle is decrease, Iron is essential to all cells and Functions of iron include involvement in energy metabolism, gene regulation, cell growth and differentiation, oxygen binding and transport, muscle oxygen use and storage, enzyme reactions, neurotransmitter synthesis, and protein synthesis (Beard, 2001). iron requirements related to rapid growth for a greater increase in blood volume, muscle mass and myoglobin (Wharton, 1999), since the iron is essential for the growth of *T. vaginalis* which is required to maintain maximal levels of ferredoxin and pyruvate-ferredoxinoxidoreductase activity (Gorrell, 1985). this may lead to decrease the iron and cause decrease In muscle mass and the result is decrease in creatinin level.

3-3 Level of inALkaline-phosphatase serum

ALkaline-phosphatase level decrease in Flagyl group (119.82 ± 10.91 c U/l), *N. sativa* 5 mg/ml (125.77 ± 9.6 b U/l), *N. sativa* 10 mg/ml (125.19 ± 16.11 b U/l), *T. foenum* 5 mg/m (85.06 ± 1.71 d U/l), *T.foenum* 10 mg/ml (56.27 ± 2.5 e U/l) , mixture *N.sativa* and *T.foenum* 5mg/ml (113.11 ± 17.9 c U/l) ,and in Control +ve(117.01 ± 0.9 c U/l) compared with Control –ve group (186.67 ± 2.11 a U/l). the difference was significant between all treatments (P<0.01) As shown in the table.(3-3)

(3-3) compare between different group in ALkaline-phosphatase.

Group	Mean ± SD
	ALkaline-phosphatase U/l
Control –ve	186.67 ± 2.11 a
Control +ve	117.01 ± 0.9 c
Flagyl	119.82 ± 10.91 c
N. sativa 5 mg/ml	125.77 ± 9.6 b
N. sativa 10 mg/ml	125.19 ± 16.11 b
T. foenum 5 mg/ml	85.06 ± 1.71 d
T. foenum 10 mg/ml	56.27 ± 2.5 e
mixture *N.sativa and T.foenum* 5mg/ml	113.11 ± 17.9 c
LSD	7.9

Similar letters mean the absence of significant differences and different letters mean the presence of significant differences at the level of probability (P<0. 01).

The amount of of ALkaline-phosphatase in the blood serum of infected mice and described in the table (4-3) shown decrease in level compared with Control –ve group (186.67 ± 2.11 a

U/l), Agree with Sharma and Honigberg(1967) Who explained the infection with *T. vaginalis* there is a progressive loss of alkaline phosphatase from both cell types; however, the acid phosphatase activity increases.Treatmentof rats with fenugreek seeds extract decreased serum acid and alkaline phosphatase, serum alanine and asparatetransferases, as well as serum amylase in diabetic rats(Shane-Mc Whorter, 2001)That explain the high decrease of ALkaline-phosphatase In infected mice that have been treated with *T. foenum* 5 mg/ml and *T. foenum* 10 mg/ml.

3.4 A comparative study between the level of aqueous extract of *N. sativa, T. foenum,* mixture *N. sativa and T. foenum* seedand metronidazole Drug in the effectiveness of *T. vaginalis* parasite.

During the 6-day swabs taken from the vagina of mice and examined under light microscope in order to know the number of trophozoite and the impact of aqueous extract on the growth of parasites and compared with the control positive and with the treatment of metronidazole, When comparing the transaction, any of them is more significant is have greater impact than controls positive we find that in general there is a variety degrees of interaction between the extract of *N. sativa, T. foenum* and mixture. In the table (3-4) shows the numbers of parasites and their impact aqueous extract during the six days and found there are significant differences between the treatments.

In the The fifth day we find that In general each transaction was a significant effect of this day level ($p < 0.01$), but the highest impact was of Flagyl and Nigella sativa concentration of 10 mg per ml as well as the treatment mixture, while the effect of treatment materials and other equal and less significant difference compared to the transactions mentioned above control of positive. Over the days, treatment mixture in general there was a significant effect by the day and the level of ($p < 0.01$), but were obtained at the highest inhibition on the sixth day of treatment. Overall, we conclude from the table that the treatment material cost, including Flagyl was of significant effect continuously exposure period six days but it was better to influence the treatment with Flagyl where he had the highest inhibition on the sixth day, as well as treatment with *Nigella sativa* 10 mg/ml and the mixture of *N.sativa and T.foenum* 5 mg/ml followed from other transactions and significantly less than 0.01 compare with positive control. figure(4-5).

The anti-trichomonal activities exhibited by those extracts were perhaps due to the possible presence of active compounds. The extracts of *N.sativa* seeds contained different classes of

alkaloids (Morikawa*et al.*, 2004) that were believed to block protein synthesis in Plasmodium falciparum (Elford, 1986). *N.sativa* seeds also contain phenolic (Nergiz and Otles, 1993).These molecules are well known for their various physiological properties, including among others, anti-parasitic, anti-inflammatory and anticarcinogenic (Ma and Kinner., 2002), Several useful pharmacological effects have been recognized to various crude and purified components of black seeds, including antihistaminergic, antihypertensive, hypoglycemic, anti inflammatory activities and mast cell stabilizing. These include immune stimulation (Swamy and Tan, 2000), anti inflammatory (Mutabagani and El-Mahdy, 1997) anti-tumor(Worthen*et al*., 1998), and anti-oxidant (Al-Awadi and Gumma, 1987).

LSD was calculated at equal or less than 0.05 / LSD FOR DAY NUMBERS: raws Expressed as (a, b ,c ,d ,e ,f ,g)
LSD FOR TREATMENT columns expressed as *,**,***,****,*****)

Number of trophozoite /ml Mean ± Sd							Treatment duration / Days
LSD	6	5	4	3	2	1	Treatment types And control
1.4	29.15 ± 2.9 a *	23.3 ± 1.6 b *	19.05 ± 1.3 c *	16.65 ± 1.8 d **	12.3 ± 0.9 e ***	12.6 ± 0.9 e ***	CONTROL +VE
1.01	0.8 ± 0.7 f ****	2.65 ± 1.4 e ***	6.55 ± 1.12 d ****	11.85 ± 0.9 c ***	14.8 ± 0.8 b **	17.65 ± 1.9 a **	Flagyl Metronidazole
1.4	11.65 ± 0.9 d **	13.95 ± 2.5 c **	13.6 ± 2 c **	18.65 ± 1.3 b **	21.6 ± 1.1 a *	22.15 ± 1.5 a *	Nigella sativa 5 mg/ml
1,2	2.6 ± 1.5 d ****	4.75 ± 2. c ***	12.3 ± 0.9 b ***	14.55 ± 0.8 a **	14.9 ± 1.5 a **	12.7 ± 1.2 b ***	Nigella sativa 10 mg/ml
1.8	12.05 ± 1.2 e **	12.25 ± 1,2 e **	15.45 ± 2.4 d **	16.6 ± 1.1 c **	19.85 ± 4.2 b *	23.75 ± 0.8 a *	Trigonella foenum 5 mg/ml
1.1	7.45 ± 1.3 d ***	13.65 ± 1.5 c **	12.9 ± 1.3 c ***	21.65 ± 1,2 b *	21.8 ± 0.9 b *	25.6 ± 1.5 a *	Trigonella foenum 10 mg/ml
0.9	3.55 ± 1.2 f ****	6.05 ± 1.1 e ***	7.4 ± 1.3 d ****	8.4 ± 0.9 c ****	21.1 ± 1,1 b *	25.55 ± 0.7 a *	mixture N.sativa and T.foenum 5 mg/ml
-------	2.3	3.02	2.8	2,6	2.1	1.8	LSD

Most of these biological activities have been attributed to thymoquinone, the main active constituent of the volatile oil extracted from the seeds (Elias *et al*., 2003). The *N. sativa* aqueous extract was found to exert an inhibitory action on the growth and motility of Trichomonasvaginalis in vitro (Tonkal, 2009), as well as a potential therapeutic effect against Blastocystishominis (El Wakil, 2007). The inhibition ability of *T. foenum*seeds aqueous extracts related to contain this plant on some vehicles Chemical, the most important alkaloids Trigonellin alkaloid, where some reports indicate that the effectiveness of this Article Against

bacteria (Majeed and Mahmood, 1988), also some study showed that crude aqueous extract of seeds Fenugreek planthave high inhibitory effect against *Brucellamelitensis* (Sultan, 1993). Another study also demonstrated the inhibitory effect of aqueous extracts of fenugreek seeds against eight types of Negative bacteria to Gram stain Solomon (Salman, 2000).

This indication show that fenugreek seeds effectiveness against bacteria give support that this extract composed from some vehicles and components that working as Antimicrobial in general and can be used against pathogenic bacteria and also against other objects, including the Parasite *Trichomonasvaginalis*, This is also confirmed by the results of the study .The results of mixing aqueous extract of *N. sativa*and *T.foenum* display a complete inhibition impact on *Trichomonasvaginalis*with high significant effect on this level ($p < 0.01$), we can conclusion from this result that the chemical compounds of*T.foenum* seeds were compliant and cooperative in the inhibitory effect of chemical compounds *N. sativa* and together have best effect on parasite than the effect of each aqueous alone. The higher activity of metronidazole at certain concentrations may be due to the fact that *N.sativa* and *T.foenum* extract was a crude extract in comparison to the raised activity of the purified metronidazole.

3-5 Histological study

3-5-1 Histological study of the uterus

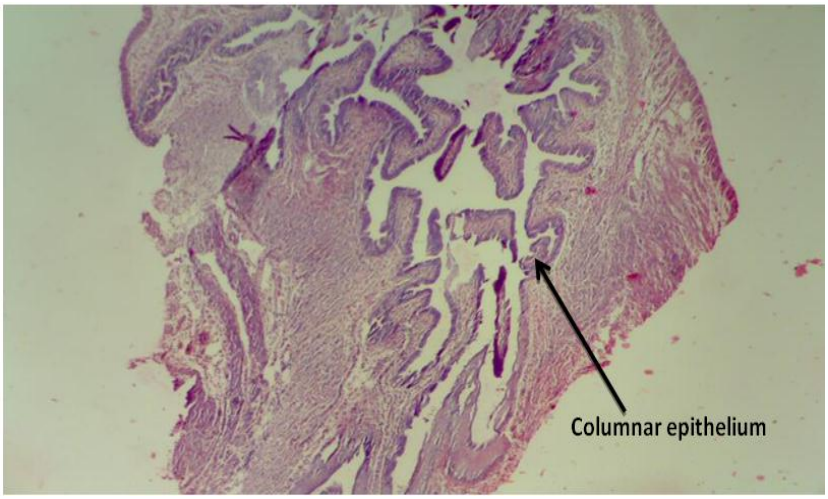

Figure: (3-1) The section of the uterus in animal control negative showing normal structures appearance lined by columnar ,non-secretory epithelium, loose connective tissue and few non-secretory gland, (H&E) (100X).

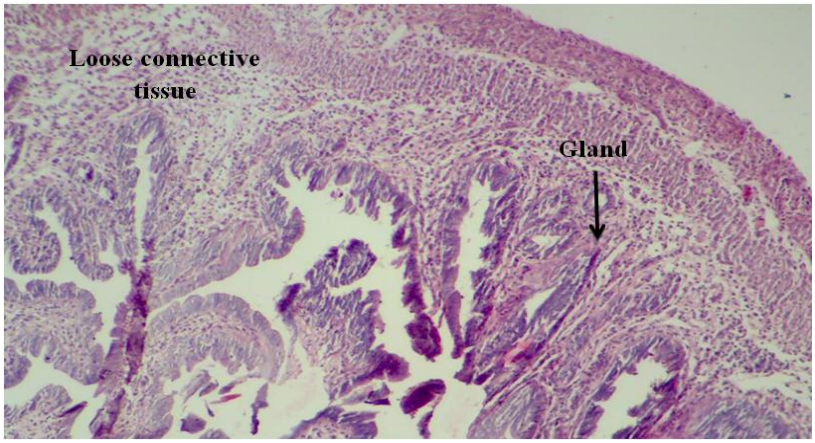

Figure: (3-2) The section of the uterus in animal control negative showing normal structures appearance lined by columnar ,non-secretory epithelium, loose connective tissue and few non-secretory gland, (H&E) (400X).

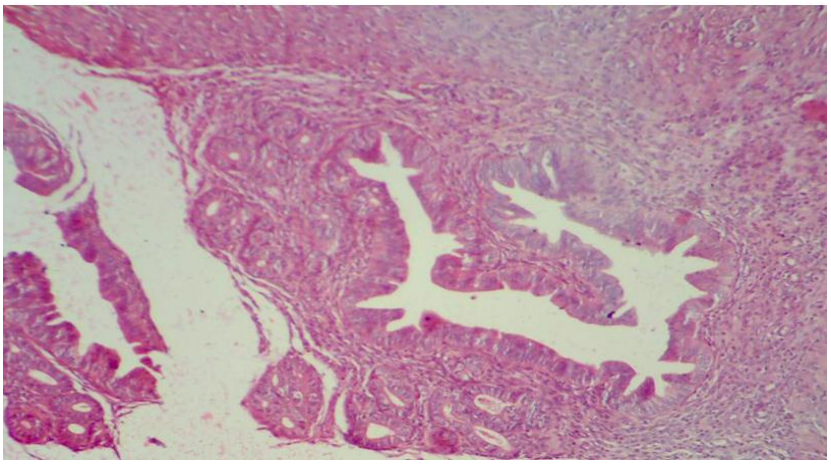

Figure: (3-3) The section of the uterus in animal control positive showing hyperplasia of lining epithelia and also the endo-gland, (H&E) (100X).

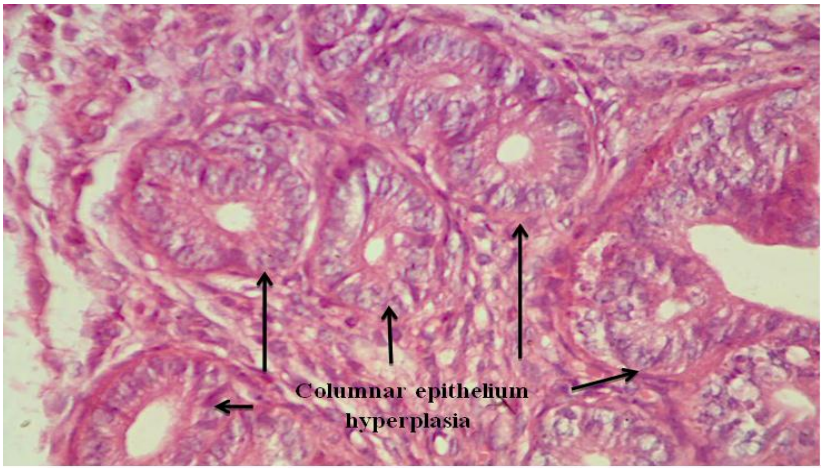

Figure: (3-4) The section of the uterus in animal control positive showing hyperplasia of lining epithelia and also the endo-gland, (H&E) (400X).

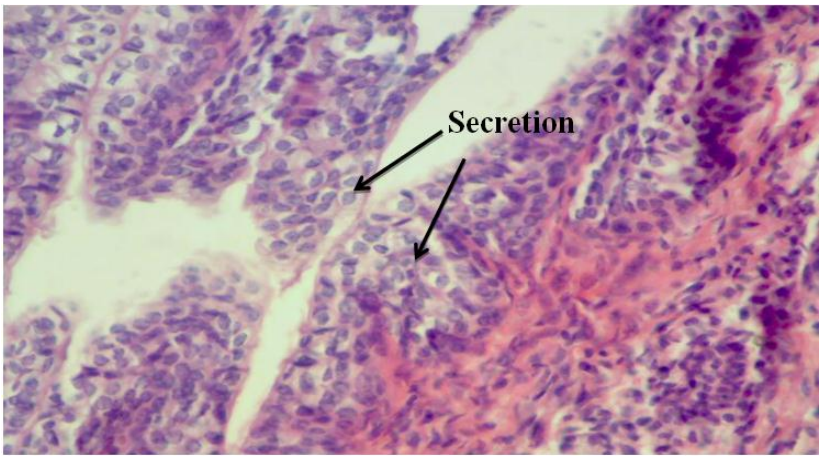

Figure: (3-5) study the effectiveness of the *N.sativa* 5 mg/ml on*Trichomonasvaginalis* in the tissue of the uterus showed slight secretory activity, (H&E) (400X).

Figure: (3-6) study the effectiveness of the *N.sativa* 10 mg/ml on*Trichomonasvaginalis* in the tissue of the uterus showed prominent secretory activity of the lining columns epithelia cell of endometrium, (H&E) (400X).

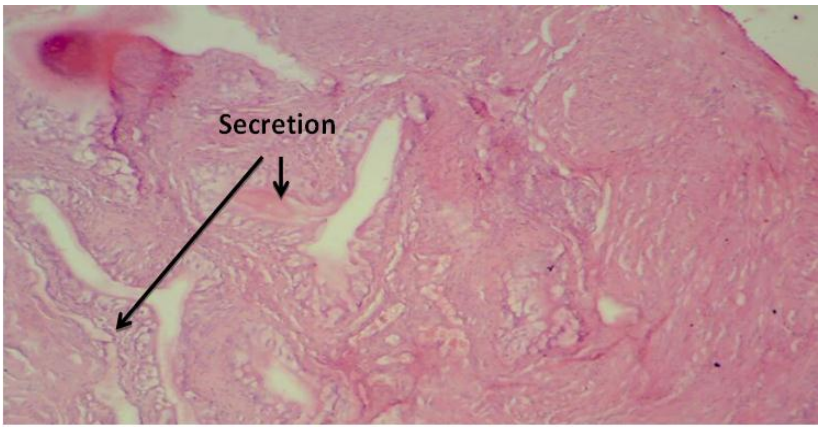

Figure: (3-7) study the effectiveness of the *T. foenum* 5mg/ml on *Trichomonasvaginalis* in the tissue of the uterus showed prominent secretory activity of the lining columns epithelia cell of endometrium, (H&E) (100X).

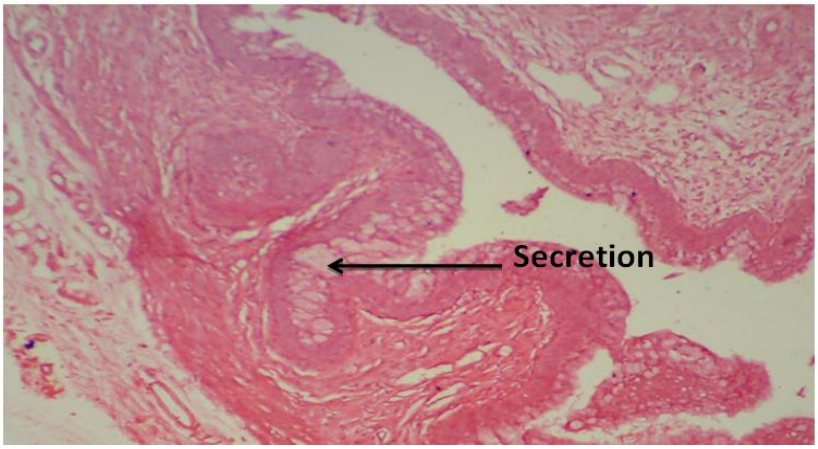

Figure: (3-8) study the effectiveness of the *T.foenum* 10 mg/ml on*Trichomonasvaginalis* in the tissue of the uterus showed prominent secretory activity of the lining columns epithelia cell of endometrium, (H&E) (400X).

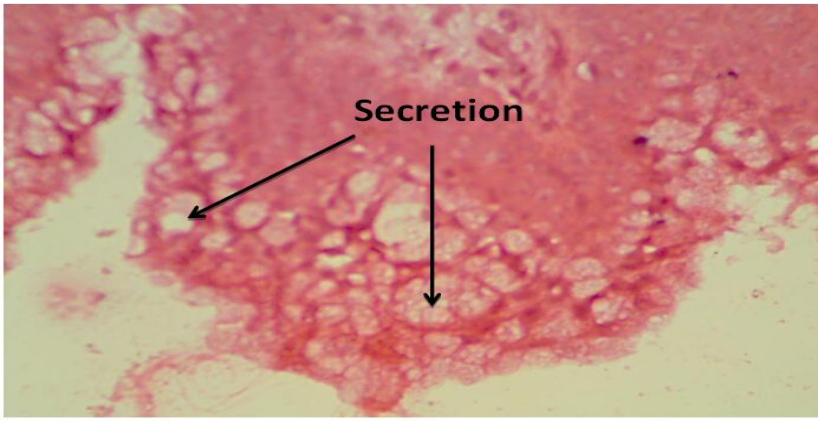

Figure:(3-9) study the effectiveness of the *N. sativa* and *T.foenum* 5 mg/ml on *Trichomonasvaginalis* in the tissue of the uterus showed prominent secretory activity of the lining columns epithelia cell of endometrium, (H&E) (400X).

Figure: (3-10) study the effectiveness of the Flagyl on *Trichomonasvaginalis* in the tissue of the uterus showed slight secretory activity, (H&E) (200X).

The endometrial tissue is inactive,it is composed of tubular glands, a dense fibroblastic stroma, and thin blood vessels. The advent of cyclic pituitary and ovarian hormonal activity results in endometrial cyclic morphologic changes involving glands, stroma, and blood vessels that can be identified as characteristic for each day of the cycle, Failure to ovulate, after menopause, first deprives the endometrium of progesteronic stimulation. The estrogenic stimulation, however, may continue because of the conversion of androgens, secreted by the menopausal ovaries and adrenal cortices, into estrogens. Obesity, diabetes, and other metabolic disorders may enhance the extragonadal endogenous estrogen production by aromatization.

High estrogen levels, especially of estradiol, are often associated with endometrial hyperplasia because of the binding of the hormone to receptor sites in the nuclei of endometrial cells (Gurpide*et al*., 1977).

Glycogen-rich epithelial cells are also transiently present in born girls who are influenced by their mother's estrogen and it is these condition which are believed to allow colonization in neonatal trichomoniasis, As soon as the effect of exogenous estrogen decreased,glycogen disappears and the pH rise, these conditions persisting until puberty (Ton Nu, P. A., 2013).

Since glucose has been found to diffuse away from the parasite under culture conditions (terKuile and Müller, 1995), That explain the increase of glucose during infection with *T. vaginalis*, and when glucose increased it will lead to glucose transport increased in the contracting muscle followed by a discussion of glycogen mobilization and synthesis by the action of glycogen phosphorylase and glycogen synthase (Thomas et al., 1996). We concluded that the increased in glucose will lead to increase the glycogen in the endometrium muscle, since the estrogen decreased the glycogen disappears so the increase of glycogen lead to increase the estrogen hormone and cause endometrial hyperplasia (Gurpide*et al*., 1977). that explain the hyperplasia of lining epithelia during *T. vaginalis* infection.

The absence of hyperplasia in infected mice that has been treated with *N. sativa*and*T. foenum* aqueous extracts resulted from the fact that these seeds can lower blood glucose and cholesterol in type 1 and type 2 diabetics and experimental diabetic animals,and Various reports have demonstrated that *Nigella sativa* crude aqueous extract have effect to restore glucose homeostasis (Labhal*et al*., 1997), and thus remains the glycogen in natural state and keeps amount of estrogen hormone and eventually prevent formation of hyperplasia.

El-Kadi and Kandil (1986) were probably the first to show that *N. sativa* seeds have immuno-potentiating properties in human T cells in vitro. This was confirmed by Haq et al. (1995), who showed that *N. sativa* seeds activate T-lymphocytes to secrete the interleukin, IL-3, and increased IL-1β production indicating a stimulatory effect on macrophages either through a direct effect or via IL-1β. In further experiments the same authors purified the proteins in the whole *N. sativa* seeds, and showed that some proteins have suppressive and others stimulatory properties in lymphocyte cultures (Haq et al., 1999). The proteins were also effective in the production of cytokines (e.g. IL-1β). The *T. foenum* seeds inhibit cancerous cells of the liver and decrease blood cholesterol level. It also has an antidiabeticproperty. Fenugreek's seed and leaf showed anticholesterolemic, anti-inflammatory, antitumor, carminative, demulcent, deobstruent, emollient, expectorant, febrifuge, galactogogue, hypoglycaemic, laxative, parasiticide, restorative and uterine tonic effects The seed includes strong mucilage which makes it a useful treatment for intestinal inflammation and ulcers(Bown,1995). These pharmacological properties as anti-inflammatory of *N. sativa* and *T. foenum* seeds may responsible for the secretory activity of the lining columns epithelia cell for the elimination of on *Trichomonasvaginalis*.

3-5-2 Histological study of the vagina

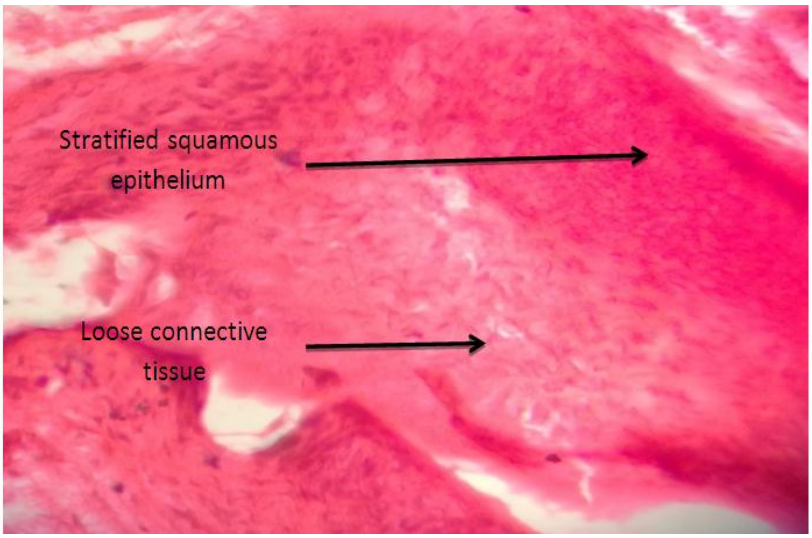

Figure: (3-11) The section of thevaginain animal control negative showing normal structures appearance lined by Stratified squamous epithelium, loose connective tissue, (H&E) (400X).

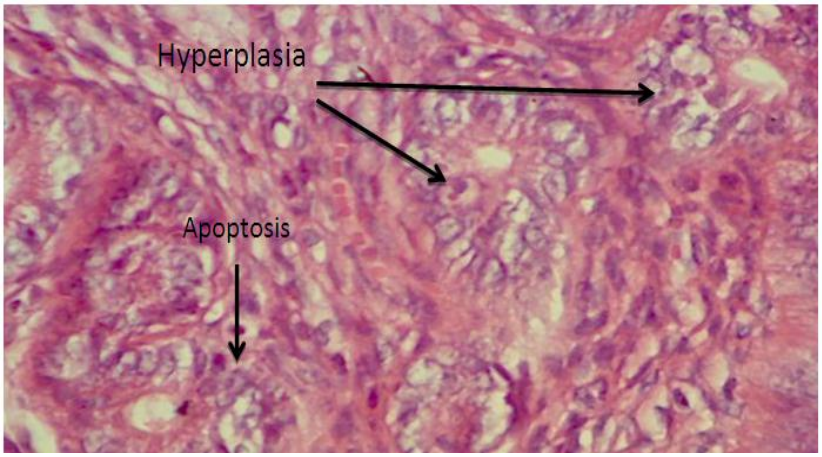

Figure: (3-12) The section of the vagina in animal control positive showing hyperplasia of lining epithelia and apoptosis(H&E) (400X).

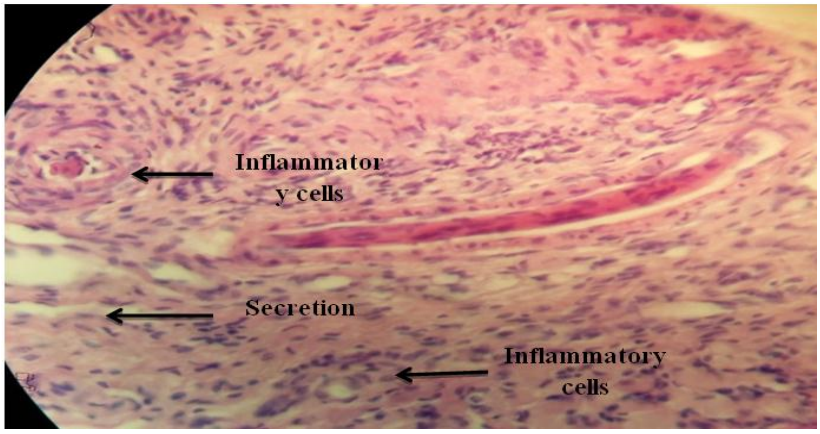

Figure: (3-13) study the effectiveness of the *N.sativa* 5 mg/ml on *Trichomonasvaginalis* in the tissue showed slight secretory activity with present of inflammatory cells (H&E) (400X).

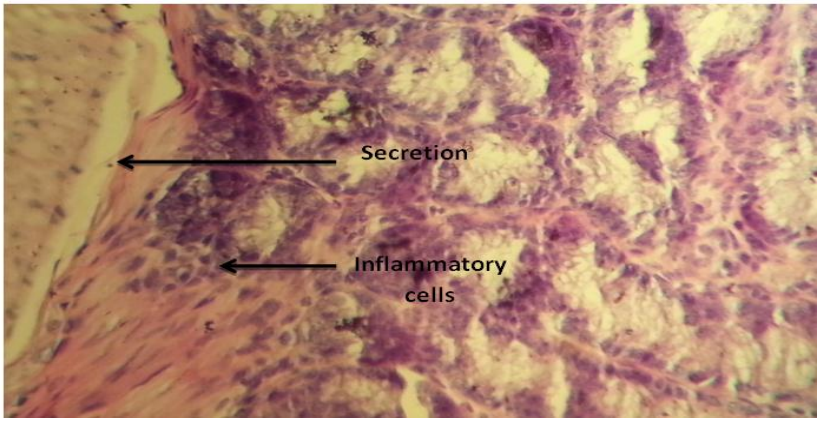

Figure: (3-14) study the effectiveness of the *N.sativa* 10mg/ml on*Trichomonasvaginalis* in the vagina tissue showed prominent secretory activity with present of inflammatory cells (H&E) (400X).

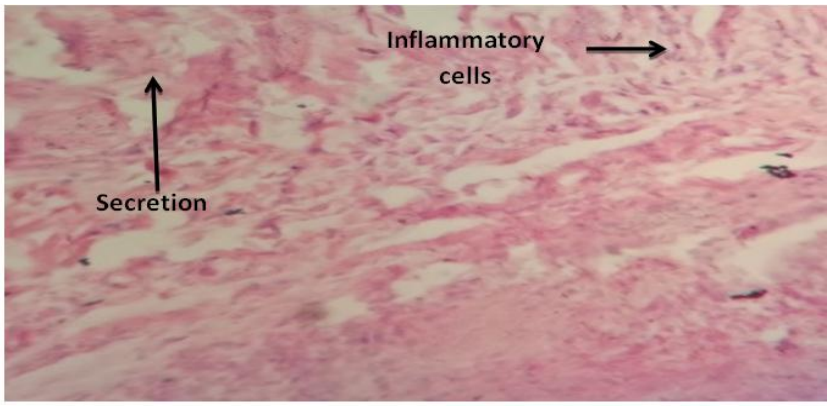

Figure: (3-15) study the effectiveness of the *T.foenum* 5mg/ml on*Trichomonasvaginalis* in the vagina tissue showed prominent secretory activity with present of inflammatory cells (H&E) (400X).

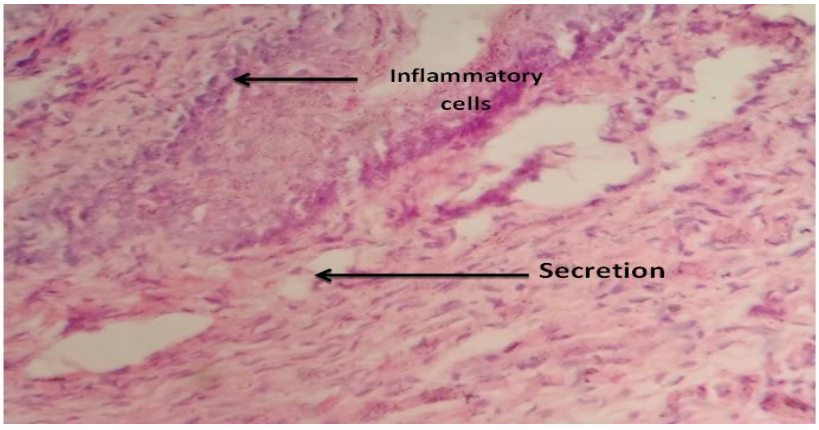

Figure: (3-16) study the effectiveness of the *T.foenum* 10mg/ml on*Trichomonasvaginalis* in the vagina tissue showed prominent secretory activity with present of inflammatory cells (H&E) (400X).

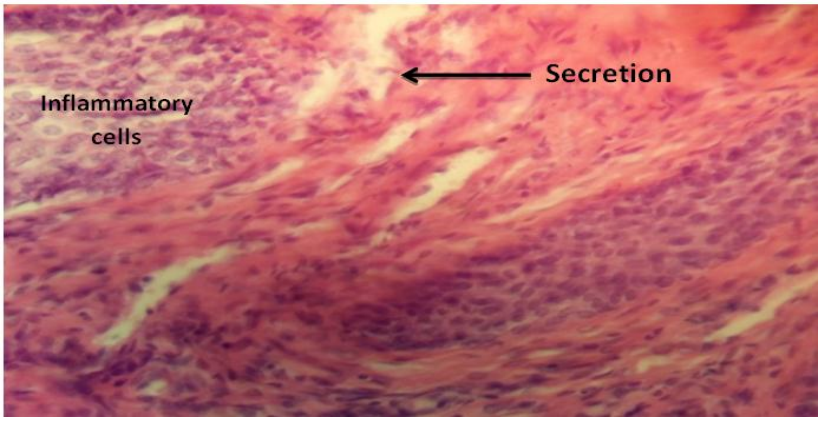

Figure: (3-17) study the effectiveness of the *theN.sativa and T.foenum 5 mg/ml* on*Trichomonasvaginalis* in the vagina tissue showed prominent secretory activity with present of inflammatory cells (H&E) (400X).

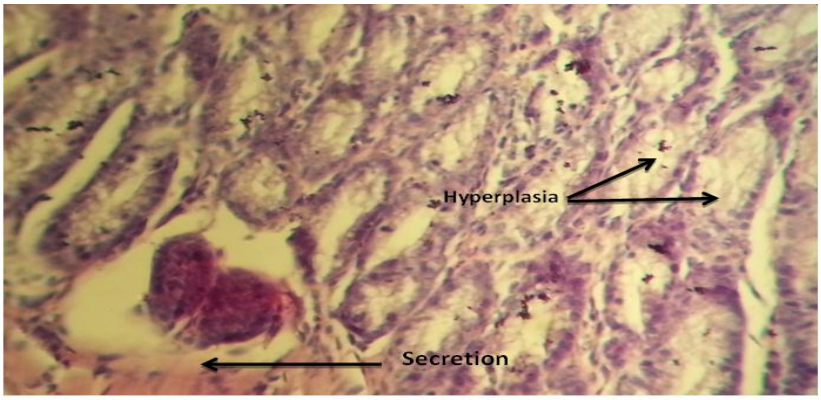

Figure: (3-18) study the effectiveness of the Flagyl on*Trichomonasvaginalis* in the tissue of the vagina showed slight secretory activity of lining Stratified squamous epithelia cell with hyperplasia, (H&E) (400X

The vagina is a fibro muscular sheath-like structure linking the external genitals with the uterus. It is lined with non-keratinizing squamous epithelium and is 8–12 cm long. The vagina protects the internal genital organs against ascending infections, forms part of the birth canal, and receives the penis in copulation.The inflammation cellular infiltration who appeared in the current study was caused by *T. vaginalis*which has been related to vaginosis, vaginitis, endometritis, adnexitis, and can activate inflammatory responses in the mucosal genital tract, increasing the risk of pelvic inflammatory disease by microhemorrhages (Seña*et al*., 2012). *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 .*T.vaginalis*evades host immunity by the presence of adhesion proteins, lipophosphoglycan ,and cysteine proteases molecules, all of which increase the pathogenicity of this intruder. The parasite adheres to the vaginal and cervical epithelial cells and triggers an immunosuppressive response from monocytes, macrophages, and dendritic cells *(*Goodman *et al*., 2011*).*

Cysteine proteinases (CP) seen to be necessary for effective adhesion protein mediated adhesion of parasites to targets. In addition, the study of Sommer in USA (2005) suggested that, CP-induced programmed apoptosis in human vaginal epithelial cells (HVEC) may be involved in the pathogenesis of *T. vaginalis* infection in vivo, may have important implications for therapeutic intervention 35.that explain the present of apoptosis in infected animal with *T. vaginalis*.

The hyperplasia who appeared in the current study was caused by *T. vaginalis* causes by the fact that *T. vaginalis* responsible for increased in glucose (terKuile and Müller, 1995), as it has been explained previously this increase will lead to increase the glycogen in the vagina muscle, since the estrogen decreased the glycogen disappears so the increase of glycogen lead to increase the estrogen hormone and cause vaginal hyperplasia (Gurpide*et al*., 1977).that explain the hyperplasia of lining epithelia during *T. vaginalis* infection.

CONCLUSIONS

1. The efficiency of Flagyl, *Nigella sativa* 10 mg/ml and the mixture of *N.sativa and T.foenum* 5 mg/ml on*Trichomonasvaginalis* more than *N.sativa 5* mg/ml *and T.foenum* 5, 10 mg/ml
2. Increasing value of glucose in all groups except in the mixture group compared with control/ve-.
3. Decreasing occur in creatinin in all group except between the Control –ve and *T. foenum* 10 mg/ml was non-significant ($P<0.01$)
4. Decreasing occur in ALkaline-phosphatase in all group compared with control/ve-.
5. *N.sativa and T.foenum*safty mild changing causing in uterus and vagina compare with Flagy.

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