

The effect of some plants extracts on *Trichomonis Vaginalis*

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Abstract:-

This is an experimental study aimed to study the effect of three plants extracts on the activity of the *Trichomonis vaginalis* parasite. After isolation of the parasite and growing on CPLM media, six plant extracts three of them were water extracts and others were alcoholic extracts which were prepared from *Artemisia herba alba*, *Punica grantum* and *Capparis spinosa* respectively were added to parasite media. Study of parasite activity and growth after 24, 48, 96, 72 hours were observed. the *Artemisia herba alba* alcoholic extract had good suppression effect through 24 hours at 0.5% concentration while the same extract from *Pumica granatum* at 0.5% concentration also affected the parasite through 48 hours and *Capparis spinosa* alcoholic extract inhibited the parasite activity in 1% concentration through 48 hours. The water extract for *Artemisia herba alba* had better inhibition effect through 48 hours at 0.5% concentration. *Punica granatum* affected through 24 hours but at 1% concentration, while *Capparis* water extract had no effect. We concluded that *Artemisia herba alba* alcoholic extract had better effect than others and we recommended further studies to extract the active substances of medical plants for the treatment of *Trichomonas vaginalis*.

تأثير بعض المستخلصات النباتية على طفيلي المشعرات المهبالية

المستخلص:-

هذه دراسة تجريبية أجريت لدراسة تأثير المستخلصات الكحولية والمائية لثلاث نباتات هي الشيح - الرمان - الكبر على طفيلي داء المشعرات المهبالية. تم تنمية طفيلي المشعرات المهبالية في الوسط الزرع (CPLM) وتم دراسة المستخلصات الكحولية والمائية للنباتات الثلاثة. أظهر نبات الشيح بتركيز 0.5% بمستخلصه الكحولي تأثيراً تثبيطياً على الطفيلي خلال 24 ساعة في حين كان تركيز 0.5% من المستخلص الكحولي لنبات الرمان هو المثبط خلال 48 ساعة. بالنسبة لنبات الكبر فقد كان تركيز 1% للمستخلص الكحولي تأثير تثبيطي خلال 48 ساعة أما المستخلصات المائية فقد كان تركيز 0.5% لنبات الشيح هو المثبط خلال 48 ساعة والرمان بتركيز 1% خلال 24 ساعة ولكن لم يظهر أي تأثير تثبيطي لنبات الكبر، وهذا التأثير التثبيطي يعود إلى وجود بعض المركبات الدوائية الفعالة التي يمكن استخدامها لعلاج مختلف الإصابات البكتيرية والطفيلية والالتهابات ولهذا ننصح بإجراء دراسات أوسع لفصل هذه المكونات المفيدة للعلاج.

Key words: *Trichomonas vaginalis*, *Artemisia herba alba*, *Punica granatum*, *Capparis spinosa*

Introduction:-

Trichomonas vaginalis infection is a common disease all over the world and its sexually transmitted disease caused by a flagellate parasite. It affects the females and affects males but usually it's asymptomatic or carriers. (1, 2, 3, 4)

In female the parasite inhabits the vaginal cavity, cervix, bartholins glands and skenes glands while in male it affects urethra, prostate, seminal vesicles, epididymis, testis and urinary bladder. (5, 6, 7)

This parasite lives in human body for long time but it doesn't live outside the body more than 24 hours especially in water (30-35min), urine and faces (30min). The parasite also affected by PH < 4.5 and >7.5, so it cannot live in normal PH of vagina (3.6 - 4.4). (3, 8, 9)

The disease is sexually transmitted, but it also can be transmitted by infected sheaths, bathrooms, internal cloths, gynecological speculums and during vaginal deliveries (from mother to new born) but these methods are not always successful because of the short life of parasite outside the human being (10, 11, 12, 13). Usually the treatment of infection with the parasite had many replaces and the parasite may be difficult to eradicate from male who transmits the disease to the female (14). Metronidazole is considered the famous drug of choice. It is bactericidal antibiotic and help to return the vaginal PH to normal (15, 16) but sometimes the vaginal secretion may protect the parasite from treatment (17). Now the WHO insist on the usage of alternative material such as medical herbal plants in the treatment of many diseases. Medical plants are defined as plants that contain active substances in its different parts such as roots, stems, leaves, fruits and seeds with little side effects on health (18). The prevalence of parasites infection among married women visiting Tikrit Teaching Hospital in Salah Al-Deen governorate was low 1.33% (as its found in the first part of this study of two parts about *Trichomonas vaginalis*) (19). This study is the second part which aim was to examine the effects of some alcoholic and water extracts of three medical plants which were *Artemia herba alba*, *Punica granatum* and

Capparis spinosa on the numbers, activity and growth of *Trichomonas vaginalis* parasite.

Materials and Methods :-**Isolation of parasite:-**

The first part of the study had 300 samples of vaginal swabs that were taken from suspected infected women who visited the outpatient of Tikrit Teaching Hospital. Normal saline (2 ml) was added to the swab. After a slide was done from each sample, it was examined by direct smear method under microscope to see the jerky and flagellate movement of the parasite (20).

Two ml of negative swab was added and cultured on prepared CPLM media from (Hi Media) which contain peptic digest of animal tissue, liver digest, maltose, L-cystiens hydrochloride and ringer's solution (pH=6±0.2) at 25°C. The preparation was done (as written by the company of the media) by adding 56 ml of media in 900ml distilled water and the preparation was heated. Then the liquid was put in 900 ml bottles and put in the autoclave (115°C). Later on 1ml of penicillin, streptomycin, nystatin and 10 ml of horse serum inactivated were added to each 90 ml of the media. Two ml from negative swab was added to the 5ml bottle of prepared media and each bottle was incubated (37 °C) to examine the parasite activity and number in 24, 48, 72, and 96 hours. (2, 7)

Medicinal plants:-

Artemia herba alba plant was collected from Al - Qadisia region in Tikrit city, *Punica granatum* plant was bought from market and *Capparis spinosa* plant was collected from Al-Aalam region. These plants were cleaned by distilled water and were dried on large filter paper and kept in dark area with continuous turn over to inhibit fungal growth. Later the plants were kept in dried well closed bottles in dark area.

Preparation of alcoholic plant extract:-

This done by harbon method (21) by dissolving 25 gm of each prepared plant in 250 ml of 70% ethanol (1:10) titer then mixed by magnetic stirrer and the fluid was filtered by Buchner funnel. The precipitate was collected and evaporated by vacuum rotary evaporator in 60°C then the fluid was filtered by whatman

no.1 filter paper to get rid of chlorophyll stain then put again in evaporator to increase its concentration. All fluid that resulted from this technique was kept in well closed dark bottles in refrigerator.

Preparation of water extract:-

This done by Al- Joboory and Al- Rawi method ⁽²²⁾ by taking 40gm of each prepared plant with 160 ml of sterile distilled water and they were mixed in magnetic stirrer and were kept in refrigerator for 24 hours then it was filtered by gauze and was filtered again by whatman no-1- filter paper .The resulted mixture put in incubator at 40 °c to increase its concentration and it was kept in dark and closed bottles.

Sterilization of alcoholic extracts:-

This is done by mixing 1 gm from extract with 5 ml of DMSO (diethyl sulphoxide) and sterilized by pasteurization.

Sterilization of water extracts:-

This is done by mixing 1 gm of extract with 5 ml of distilled water and sterilized by membrane filter (0.22µg) to inhibit bacterial and germs to pass from it.

After sterilization, from each water and alcoholic extract five concentrations were done (0.20 %, 0.25 %, 0.50 %, 0.75%, 1%) and we dissolved the alcoholic and water extract in (0.20, 0.25, 0.50, 0.75, 1) gm/ml with the media. After growing the parasite in culture media, the bottles which contained the media and plant extracts were inoculated with the parasites .The parasite was added also to another bottles which did not contain the plant extract, only contained the media to be a negative control .The parasites numbers and movement activity were examined in 24, 48, 72, 96, hours to find the concentration affects the parasite activity and growth.

The effect degree of plants extracts concentrations on the parasites were denoted as follows:

+++ (active movement=it means the parasites were in binary division and some of them did not complete their division)

nm (normal movement= it means the parasites completed their binary division)

++ (weak movement= it means the parasites started death stage by decreasing their numbers and movement).

+ (very weak movement= it means more decreasing in parasites numbers, movement and the flagellate movement became very slow)

_ (absence=complete death of the parasites)

We used F test and the least significant difference LSD in statistical analysis of the study.

Results:-

The numbers and movement types according to the observation time in the negative control bottles show in table (1).The results also showed different effects according to the type of plant extract, and they were as follows:-

1-Artemisia herba alba:-

The 0.50% concentration of alcoholic extract showed complete cessation of parasitic activity at 24 hours while the 0.25 % concentration showed complete cessation after 48 hours as shown in table (2) and the 0.20% concentration had significant difference from others effects .

Table (3) showed the effect of water extracts at 0.75 % concentration which occurred through 24 hours while the 0.50% and 0.25% concentrations showed the same effect but through 48 and 96 hours in consequences. These effects were not significant except between the 0.20 % concentration and all others excluding 0.25 % concentration.

2-Capparis spinosa:-

Table (6) showed that 0.75% and 1% concentrations of alcoholic extract were the only two concentrations that inhibited the activity through 48 hours and 96 hours in consequence while the others had no effects. The water extract had no effect on parasite at all concentrations, table (7). The results of table (6) and (7) were not significant.

3-Punica granatum:-

Table (4) showed the effect of alcoholic extracts and 0.75 % concentration had total inhibition of parasite activity and growth through 24 hours while the 0.20% concentration had no effect in comparison to negative control, table -3- results were significant except between 0.20% and 0.25% concentrations.

While table -4- showed that water extract at 1 % concentration had total inhibition through 24

hours, the results of table (5) was not significant.

Table (1) The numbers and movement type according to the time (negative control)

Time of observation	Movement of parasite	Mean of parasite numbers
24hours	+++	945000
48hours	Nm	716000
72hours	++	488000
96hours	+	94000
120hours	-	000000

Table (2) The effect of *Artemisia herba alba* alcoholic extract on the *Trichomonas vaginalis* parasite

Time Concentrations	24hrs.	48hrs.	72hrs	96hrs
% 0.20	+++	Nm	++	+
% 0.25	+	-	-	-
% 0.50	-	-	-	-
% 0.75	-	-	-	-
% 1	-	-	-	-

Table (3) The effect of *Artemisia herba alba* water extract on the *Trichomonas vaginalis* parasite

Time \ Concentrations	24hrs.	48hrs.	72hrs	96hrs
0.20%	+++	Nm	++	+
0.25%	nm	++	+	-
0.50%	+	-	-	-
0.75%	-	-	-	-
1%	-	-	-	-

Table (4) The effect of *Punica granatum* alcoholic extract on the *Trichomonas vaginalis* parasite.

Time \ Concentrations	24hrs.	48hrs.	72hrs	96hrs
% 0.20	+++	Nm	++	+
% 0.25	nm	++	+	-
% 0.50	+	-	-	-
% 0.75	-	-	-	-
% 1	-	-	-	-

Table (5) The effect of *Punica granatum* water extract on the *Trichomonas vaginalis* parasite

Time \ Concentrations	24hrs.	48hrs.	72hrs	96hrs
0.20%	+++	Nm	++	+
0.25%	+++	Nm	++	+
0.50%	nm	++	+	-
0.75%	+	-	-	-
1%	-	-	-	-

Table (6) The effect of *Capparis spinosa* alcoholic extract on the *Trichomonas vaginalis* parasite

Time Concentrations	24hrs.	48hrs.	72hrs	96hrs
% 0.20	+++	Nm	++	+
% 0.25	+++	Nm	++	+
% 0.50	++++	Nm	++	+
% 0.75	nm	++	+	-
% 1	+	-	-	-

Table (7) The effect *Capparis spinosa* water extract on the *Trichomonas vaginalis* parasite

Time Concentrations	24hrs.	48hrs.	72hrs	96hrs
0.20%	+++	Nm	++	+
0.25%	+++	Nm	++	+
0.50%	+++	Nm	++	+
0.75%	+++	nm	++	+
1%	+++	nm	++	+

Discussion:-

Treatment of *Trichomonas vaginalis* depends on return of vaginal pH and inhibition of activity and growth of parasite either by local or oral treatment⁽¹⁴⁾. The first plant is *Artemisia herba alba* which called in English language worm wood. It is a medical herbal plant. There is many types of it. The common one that is found in our area is *Artemisia herba alba*^(23,24). There are many active medical compound that extracted from this plant according to its type and the most known compounds are santonine, alkyl disulphide, guinolizidine, guanidine^(24,25,26). The effect of *Artemisia* extracts on parasite was better with little time (24 hours) and this agreed with Al- Tae (2000)⁽²⁶⁾. This may be due to its active substances such as volatile oil, lipophilic flavonoid, resin

compounds and ketones^(27, 28). This plant is used for many purposes such as the treatment of chest infection, intestinal worms, diabetic mellitus, antimalarial drug, antibacterial and antifungal drugs^(23, 28). The second plant is *Punica granatum* which is shrub plant from fruit group. It contains carbohydrates, fat, fibers, calcium, phosphorus, ferrous, vitamin B, vitamin C, citric acid, estrogen, gallo-tannin and tannins. The extract of *Punica* can be used as germicidal especially in vaginal infection and treatment of some intestinal worms.^(29, 30)

Punica granatum effects might be due to inhibition effect of *Punica* peels which contain glycosides and gall of pelletierine and it can kill many germs and parasites. So the peel of sap is used in the treatment of different vaginal

secretions and infections because of its fungal and bacterial growth inhibition. ^(27, 29, 30, 31, 32)

The third plant in the present study is *Capparis spinosa*. It's a woody herbal plant ⁽²³⁾. The active compounds which extract from it are rutin , rutiic acid , rectic acid , saponin , quaternary ammonium , and three antiseptic substance which is cappapernal from alcoholic extract of plant ^(28,32). It used as antipyretic , anti inflammatory , germicidal , increase urine output and increase platelet aggregation. So it is used in urinary tract infection, anti diarrheal, wound antiseptic, eye infection, and decrease anal fissure bleeding. ^(29, 33, 34)

Capparis spinosa can affect the parasite activity and growth because it contains glycosides which have antiseptic effect on germs and it can treat different infection. ⁽³⁴⁾

Conclusions:-

We concluded that there is many medical plants extracts that contain active substances which inhibit the growth of parasite especially *Artemisia herba alba* extracts. The alcoholic extracts had better effects than water extracts on parasite activity and growth.

Recommendations:-

We recommended further studies to separate the different active substances of these medical plants, so they can be used as alternative treatment for *Trichomonas vaginalis* with little side effects.

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