Effect of Aqueous Extract of Garlic *(Allium sativum)* on *Trichomonas gallinae* in Pigeons

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Abstract

The present study aimed to investigate in vitro and in vivo effects of aqueous garlic extract (AGE) on *T. gallinae* growth and multiplication in comparison to metronidazole (MTZ). Concentrations of AGE (200, 100, 75, 50 and 25 mg/ml) and of MTZ (12.5, 25, 50, 75 and 100µg/ml) were dissolved in incubation medium. Growth of *T. gallinae* at 37°C was observed after 24, 48 and 72 hrs. Efficacy of AGE and MTZ on trophozoites and minimal lethal concentrations (MLC) were determined.

Inhibition of parasite growth occurred in proportion to concentration of AGE and incubation time. MLC of AGE was 75, 50 and 50 mg/ml after 24, 48 and 72 hrs, respectively. Growth reduction of parasite was 43.24%-88.89% in all incubation periods. Similar effects were obtained with MTZ, as its MLC was 50, 25 and 12.5 µg/ml after 24, 48 and 72 hrs, respectively.

During treatment with AGE (200 mg/Kg BW), population density of *T. gallinae* was decreased reaching the lowest degree on 4th day and became 0 with 100% recovery of birds on 5th day. Meanwhile, during treatment with MTZ (50 mg/Kg BW), its population was decreased to the lowest degree from 4th till 6th day of treatment and became 0 with 100% recovery on 7th day. RBCs count and Hb%, total protein, albumin and globulin in squabs treated with AGE and MTZ were increased and directed towards normal level. Whereas, total WBCs, differential counts, AST, ALT and cholesterol were decreased in AGE- and MTZ-treated groups comparable to control. At 4th week PT with AGE, body weight of birds was increased compared with MTZ-treated group. Leukocytic aggregations in crop with severe hydropic degeneration of hepatocytes in control, mild degeneration in hepatocytes in AGE-treated and hydropic degeneration in hepatocytes of MTZ-treated birds were seen.

It could be concluded that garlic had a highly inhibitory effect on *T. gallinae*, as that of MTZ. This suggests that garlic may be promising phytotherapeutic agent for trichomoniasis and regains liver of infected pigeons to almost normal appearance.

Key words: *T. gallinae, trophozoites, MTZ-treated, hepatocytes .*

Introduction

Trichomonas gallinae (flagellated protozoan) usually inhabits upper gastrointestinal tract of different avian species, especially pigeons allover the world and causes ulcers (condition known as "canker") **(Qiu *et al*., 2012)** and enters circulatory system reaching liver where they cause formation of caseous lesions leading to serious losses and high mortality especially in young birds **(Narcisi *et al*., 1991)**. T. gallinae was transmitted from adults to squabs through milk and infection was done within minutes **(McDougald, 2003)** and increased thickness of crop wall due to presence of hyperkeratinization of mucosa **(Abd El Rahman, 1991)**. In liver, abscesses with inflammatory reactions were detected **(Narcisi *et al*., 1991)**.

Several drugs were active against trichomoniasis in pigeons. Metronidazole (MTZ) has so far been the most widely applied drug for treating trichomoniasis. MTZ was 100% effective in naturally infected pigeons with T. gallinae **(Abd EL-Motelib and Galal, 1993 and Aydin *et al*., 2000)**. Also, it has 100% efficacy against *T. muris* in mice when compared with

garlic extract **(Elmadawy, 2006)**. Almost MTZ had sideeffect in dogs and increased Candida growth in gastrointestinal tract **(Dow *et al.*, 1989)** and led to drug resistance and potential risks of carcinogenicity **(Sobel *et al.*, 1999)**. Therefore, new anti-protozoal drugs with high effectiveness and low toxicity are required for treatment of these parasitic protozoa. So, medicinal plants can be an alternative resource for novel anti-protozoal drugs **(Arthan *et al.*, 2008)**.

AGE had a good efficacy against *T. muris* in mice **(Elmadawy, 2006)**. Garlic was safe treatment of other protozoan parasites as Giardia sp, Entamoeba histolytica, Trypanosoma lewisi, Histomonas meleagridis and Cryptosporidia by **Soffar and Mokhtar (1991), Lun *et al.* (1994), Nok *et al.* (1996), Zenner *et al.* (2003) and Wahba (2003)**. Prophylaxis of AGE in challenged rabbits with E. stiedae had better body gain **(Abu-Akkada *et al.*, 2010)**. In recent years, a few studies have been performed on effects of AGE on Aspiculuris tetraptera in mice **(Ayaz *et al.*, 2008)**.

Therefore, this work was carried out to investigate in vitro and in vivo activity of AGE (*A. sativum*) on growth and multiplication of T. gallinae in pigeons compared with MTZ besides some biochemical, haematological and pathological studies.

Materials and Methods

Samples were collected from lesions and caseated mass of diseased pigeons and examined by high power for detection of T. gallinae. Slides were prepared, stained with 10% Giemsa stain and examined under oil immersion lens **(Levine, 1985)**.

Swabs were prepared from lesions and dipped into test tubes containing culture media (Glucose-serum broth medium) (GSB) at pH 7 **(El-Sayed, 2005)**. The tubes were incubated at 37°C for 7 days and cultures were counted according to **Helmy (1995)** using a measuring dropper (giving 3 drops / 0.1 ml) to adjust inoculating dose of T. gallinae, and to evaluate effect of AGE and MTZ on their growth.

Aqueous garlic extracts (AGE) were prepared according to **Gharavi *et al.* (2011)** as follows: 200 gm of peeled cloves of aged garlic bulbs (*A. sativum*) were ground, minced in small pieces by electric blender and shacked with 100 ml of sterile distilled water on a shaker for 48 hours at 37 °C. This mixture was centrifuged at 2000 rpm for 25 min. Supernatant was filtered through Whatman (size 1) filter paper and kept frozen at -20 °C as a stock solution until use.

In vitro, concentrations of AGE were selected after several trials aiming to reach that which would produce total inhibition of growth after 24 hrs. AGE was dissolved in GSB medium forming 5 conc. (200, 100, 75, 50 and 25 mg/ml). Growth was observed after 24, 48 and 72 hrs at 37 °C. Metronidazole; MTZ; (Flagyl® suspension) was dissolved in GSB medium forming 5 conc. (12.5, 25, 50, 75 and 100 µg/ml) according to **Ali (2007)**. MTZ-treated and control cultures of parasites were submitted to the same procedure used for AGE culture. Four tubes were used for each concentration of AGE and MTZ and control cultures. Minimal lethal concentration (MLC) was calculated as the lowest conc. of tested AGE and MTZ, at which no parasite was observed **(Meingasser and Thrner, 1979)**.

Seventy three, (25+48) apparently healthy squabs up to 3 weeks of age were obtained from domesticated pigeons of Kafr Aly village, Kaliubia governorate, Egypt. Squabs were subjected to parasitological examination to assure that the examined squabs were free from infection.

In experiment 1, twenty five squabs were divided into five groups (5 squabs per each) and reared in separate wire cages. First group was left as a negative control (not infected and not treated). While each squab of other groups was inoculated with $1x10^4$ of 48 hrs cultured T. gallinae trophozoites using stomach tube **(Stabler, 1975)**. Second group was left as a positive control (infected and not treated). However, at the same day of infection, third, fourth and fifth groups were treated with AGE at concentration of 1/10, 1/20 and 1/40 from stock solution respectively (i.e, 200, 100 and

50 mg/kg BW) in drinking water for seven successive days.

In experiment 2, forty eight squabs were divided into four groups (12 squabs per each), allocated in wire cages by ranking method and fed balanced ration of grains (beans, corn, wheat and rice). At day 0 (day of infection), first group was not infected and not treated. The remaining groups were experimentally infected with isolated T. gallinae strain. Each squab received $1x10^4$ of 48 hrs cultured T. gallinae trophozoites (**Stabler, 1975)**. Second group was infected and not treated. Meanwhile, at the same day of infection, third group was administered daily with AGE (200 mg/kg BW) for seven successive days in drinking water **(Elmaday, 2006)**. Fourth group was administered daily with MTZ (Flagyl® suspension, Sanofi Aventis, Egypt) (50 mg/kg BW) for seven successive days in drinking water (producer). Treatment started at the same day of infection. Numbers of living trophozoites were daily counted from crop under microscope per High Power Field (HPF) and population density were scored **(Diamond, 1954)** and growth inhibition percent was calculated **(Palmas *et al*., 1984)** as following equation:

$$\text{Growth inhibition \%} = \frac{\text{Mean no. of trophozoites in control group - Mean no. of trophozoites in test group}}{\text{Mean no. of trophozoites in control group}} \text{ X } 200$$

Population density of living T. gallinae in crop of squabs treated with AGE or MTZ was estimated daily for 7 successive days **(Diamond, 1954)**. Responded birds to treatment were recorded. Two blood samples were collected from wing veins of 5 squabs in each group two weeks PT. First part was collected on heparine for hematology as erythrocytic and total leucocytes counts **(Schalm, 1986)** and haemoglobin (Hb%) **(Wintrobe, 1965)**. Second part was allowed to separate serum for estimation of total proteins **(Sonnenwirth and Jarett, 1980)**, albumin **(Beng and Lim, 1973)**, globulin and aspartate aminotransferase (AST) and alanine amino-transferase (ALT) **(Reitman and Frankel, 1957)**. Body weight was recorded at day of infection and weekly as well as mortalities along experimental period. Specimens were obtained from crop and liver of infected squabs and fixed in formal saline 10%, passed in alcohols and xylene and embedded in paraffin wax. Sections of 5-7 microns thickness were prepared, stained with H and E and examined **(Bancroft and Gamble, 2002)**. Data obtained in this study were analysed **(Duncan, 1955)** using computer program **SPSS (2001 Ver., 11)** and means were compared at significance of 0.05%.

Results and Discussion

Trichomoniasis is a common disease in pigeons, causing high losses among squabs. Its severity and mortality in squabs may be attributed to their high susceptibility or to highly pathogenic strains of T. gallinae **(Mc Dougald, 2003)**.

Morphological feature of T. gallinae isolated from crop of naturally infected squab was flagellated, pear shape and dimensions of body were averaged 11.12µm ±0.035 x 8.14µm ±0.017, (n=10) for fresh form of T. gallinae (Fig, 1-A). These features of isolated T. gallinae are nearly similar to that described by **Soulsby (1986)**. Site of infection was observed in gastrointestinal tract from mouth to crop and in other organs as liver. Similar results were reported by **Abd El-Rahman (1991)**. However other authors demonstrated lesions in other organs as spleen **(Helmy, 1995)**; liver, brain and pericardium **(El-Sayed, 2005)**. Differences in predilection sites may be due to differences in strains of parasite or due to different localities.

In this study, clinical sings of infected pigeons were dullness, inability to eat, drink and fly, opened beak with offensive odour and fluid from mouth. Grossly, infected pigeons showed yellowish white caseated material varying in size in buccal cavity (Fig., 1-B). These results are in agreement of that of **Abd El-Motelib and Galal (1993) and Helmy (1995)**. Liver of

some pigeons was dark red enlarged with small yellowish necrotic patches (Fig, 1-C). Parasites reached such organ may be attributed to differences in virulence of strains similarly as suggested by **McDougald (2003)**.

Regarding to *T. gallinae*, in vitro effects of garlic as sources of allicin have not been previously reported. Thus, present study was carried out to investigate in vitro effects of AGE on growth and multiplication of T. gallinae compared to standard drug MTZ. Although MTZ was very effective, AGE was also shown to have a statistically highly significant effect on multiplication of *T. gallinae*. Inhibition of parasite multiplication depends on concentration of drug and incubation time. MLC of AGE was 75, 50 and 25 mg/ml after 24, 48 and 72 hrs, respectively (Table 1), although the lowest concentration of 25 mg/ml AGE after 72 hrs failed to cause total inhibition of multiplication, it showed growth reduction by 43.24-88.89 % in all incubation periods (Table 1). Similar effects were obtained with MTZ, as its MLC was 50, 25 and 12.5 µg/ml after 24, 48 and 72 hrs, respectively (Table 2). AGE was similar to MTZ in inhibiting growth and multiplication of T. gallinae (Tables 1 and 2). These results agreed with that of **Ahmed (2010)** on T. vaginalis, **Harris *et al.* (2000)** on Giaridia and **Zenner *et al.* (2003)** on Tetratrichomonas gallinarum and Histomonas meleagridis in vitro. Experimenting with *T. vaginalis*, Persian shallon (A. hirtifolium) which is a native plant in Iran, showed in vitro antitrichomonal activity at low concentrations and in short times **(Taran *et al.*, 2006)**. With garlic also, **Polat *et al.* (2008)** reported that methanol extract of *A. sativum* has amoebicidal effect on Acanthamoeba castellanii trophozoites and cysts.

Main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin reductase and RNA polymerase. These can affect the essential metabolism of cysteine proteinase activity involved in virulence of trichomonad parasite **(Ankri and Mirelman, 1999)**, as well as its specific reaction with free sulfhydryl group present in active site of cysteine proteinase of parasite **(Rabinkov *et al.*, 1998)**. Experiments with E. histolytica have shown that pure allicin inhibits parasite's cysteine proteinase leading to cytopathological effects associated with infection **(Ankri *et al.*, 1997)** and growth of parasite **(Mirelman *et al.*, 1987)**. While cysteine proteinases are present in a wide range of parasitic protozoa including Trichomonas and appear to be relevant in several aspects of life cycle and parasite-host relationships **(Gradoni and Ascenzi, 2004)**. Furthermore, some of breakdown products of allicin have ability to cross cell membranes and combine with sulphur-containing molecular groups in amino acids and proteins, thus interfering with cell metabolism **(Davis, 2005)**.

Results of present study and those of other researches are growing evidence of antitrichomonal activity of garlic. Present study recorded greater activity of garlic against T. gallinae as its MLC was 75 mg/ml. This result agrees with reports of several studies **(Amin and Kapadnis, 2005 and Ahmed, 2010)**. Garlic (200 mg/kg BW) had the heighest anti-trichomonal effect and shortened course of treatment in pigeons from 7 to 5 days. Zero population density and 100 % recovery of birds occurred on 5th day of treatment (Table 3). Similar results were reported by **Elmadawy (2006)** who applied AGE in mice infected with *T. muris*.

During treatment, count and population density of *T. gallinae* of living parasite in crop gradually decreased reaching the lowest degree (+) in AGE-treated group at 4th day of treatment, but starting from 4th to 6th day in MTZ-treated one, and it became (0) on 5th day and 7th day of treatment in treated groups with AGE and MTZ, respectively (Table 4). Concerning AGE -treated group, our results are in agreement with findings of **Elmadawy (2006)** who reported that AGE had a good efficacy (90.5%) against *T. muris* in mice, delayed its prepatent period and shortened the course for disease. In present study, garlic extracts shortened duration of treatment from 7 days to 5 days. Similar results were reported by **Soffar and Mokhtar (1991)** who found that garlic *A. sativum* aqueous extract used in 2 doses per day for 3

days in children infested with H. nana and Giardia spp was efficient, safe and shortened the duration of treatment. Regarding to MTZ-treated group, in our study, count and population density of living parasite in crop of infected pigeons and treated with MTZ decreased gradually till 6th day of treatment and become zero on 7th day. Similar results were reported by **Helmy (1995)** and **El-Sayed (2005)**.

In present study, recovery of birds after treatment was 100 % on 5th and 7th day of treatment with AGE and MTZ respectively. Oral administration of AGE (200 mg/Kg BW) for seven successive days resulted in 100% recovery after only 5 days PT (shortest treatment course) (Table 4). Similar results were reported by **Elmadawy (2006)**. While administration of MTZ (Flagyl) (50 mg/Kg BW) for seven successive days resulted in 100% recovery after 7 days PT (longer treatment course). These results are in agreement with that reported by many authors **(Fouly, 1990; Helmy, 1995 and El-Sayed, 2005)**. On the other hand, efficacy of MTZ was 100 % in T. gallinae infected pigeons (100 mg/kg BW) for 7 days **(Ayden *et al*., 2000)**.

In present study, hematological studies revealed that positive control squabs showed macrocytic hypochromic anaemia (Table 5). These results are in agreement to findings obtained by **El-Sayed (2005)**. Treated groups with AGE and MTZ showed a recovery from anaemia after 14 days PT similar to results reported by **Shihata (1978)**. Lymphocytosis, heterophilia, eosinophilia, basophilia and monocytosis were observed in infected pigeons with T. gallinae. Similar results were reported by **Shihata (1978); Narcisi *et al*. (1991)** and **El-Sayed (2005)**. Eventually, in our study, blood parameter levels would return to normal level after AGE and MTZ treatments.

Serum total protein, albumin and globulin were significantly increased in treated birds with AGE and MTZ when compared with positive control. Meanwhile AST and ALT activities and total cholesterol were significantly decreased in infected and treated groups if compared to infected control one (Table 6). The increase in AST and ALT levels in infected squabs could be due to liver damage produced by pathogenic strain of T. gallinae that invaded liver. These results are similar to that of **Galab (1994)**. In treated pigeons with AGE, total protein, albumin and globulin began to return to normal level (2 weeks PT) faster than MTZ (3 weeks PT) (Table 6). This may be attributed to its rapid antitrichomonal effect as that reported by **Ahmed (2010)**. In addition, it may rapidly enhance regeneration of hepatocytes if compared to MTZ. Meanwhile AST, ALT and cholesterol were decreased in AGE-treated group compared to MTZ (Table 6). These results agreed with that of many authors **(Toulah and Al-Rawi, 2007; Abu-Akkada *et al*., 2010 and Dkhil *et al*., 2011)**. Garlic lowered cholesterol in chickens **(Qureshi *et al*., 1983 and Kaya and Macit, 2012)**. However garlic and silymarin have synergestic effects as hepatoprotective against hepato-toxicity in rats **(Sener *et al*., 2005 and Shaarawy *et al*., 2009)**.

At 4th week PT, body weight of pigeons treated with AGE was increased when compared with MTZ and control. However MTZ group was similar to control (Table 7). These results agreed with that of **Abu-Akkada *et al*. (2010)** who found that a prophylactic dose of garlic extract improved body weight in challenged rabbits with E. stiedae. **Tatara *et al*. (2008)** reported that AGE induced beneficial effects on health status, performance and systemic development of piglets. Mortality percent was 8.33% in AGE-treated, 16.67% in MTZ-treated and 41.67% in positive control groups (Table 8). This may be attributed to effect of AGE against Trichomonas, *Candida albicans* and bacteria besides its immunostimulant and hepatoprotective effects **(Dkhil *et al*., 2011)**. Meanwhile, MTZ increased Candida growth in pigeon crop besides its drug resistance. Similar results were reported by **Dow *et al*. (1989)** in dogs.

Crop of infected pigeons with T. gallinae showed necrosis and focal submucosal leukocytic aggregations (Fig., 2-A) and vacuolation of mucosal epithelium (Fig., 2-B). Similar re-

sults were reported by **Mohamed *et al.* (2009)**. Liver of normal squabs showed normal architectures (Fig., 2-C). Liver of positive control showed necrosis and severe hydropic degeneration in hepatocytes (Fig., 2-D) similar to that reported by **Mohamed *et al.* (2009)**. Meanwhile, AGE-treated livers showed mild degenerative changes in hepatocytes with almost normal appearance (Fig., 2-E). These results agreed with many authors **(Toulah and Al-Rawi, 2007; Abu-Akkada *et al.*, 2010 and Dkhil *et al.*, 2011)**. However, liver of MTZ-treated birds showed hydropic degenerative changes in hepatocytes (Fig., 2-F).

Conclusion

It is concluded that garlic is as efficient as MTZ in inhibiting growth of T. gallinae trophozoites in culture and in infected pigeon, possessing more advantage of being a natural product. It showed improvement of some haematological, biochemical and economical parameters. So, we recommended using garlic at a dose of 200 mg/kg BW for treatment of trichomoniasis in pigeon without sideeffect.

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Table (1). In vitro mean count and percentage of growth inhibition of T. gallinae per culture (trophozoite number x 10^4) after exposure to various concentrations of AGE in comparison to non treated control (Mean ± SE, n = 4)

Time after treatment in vitro	Mean ± SE and Growth inhibition %	Concentrations of aqueous garlic extract (AGE)						LSD
		None treated control	25 mg / ml	50 mg / ml	75 mg / ml	100 mg / ml	200 mg / ml	
24 h	Mean ± SE	9.25 ^{a} ± 0.25	5.25 ^{b} ± 0.25	3.00 ^{c} ± 0.41	0.00 ^{d} ± 0.00	0.00 ^{d} ± 0.00	0.00 ^{d} ± 0.00	2.25 *
	Growth inhibition %	0.00 %	43.24 %	67.57 %	100 %	100 %	100 %	-----
48 h	Mean ± SE	7.00 ^{a} ± 0.41	2.75 ^{b} ± 0.63	0.00 ^{c} ± 0.00	0.00 ^{c} ± 0.00	0.00 ^{c} ± 0.00	0.00 ^{c} ± 0.00	2.75 *
	Growth inhibition %	0.00 %	60.71 %	100 %	100 %	100 %	100 %	-----
72 h	Mean ± SE	2.25 ^{a} ± 0.25	0.25 ^{b} ± 0.25	0.00 ^{b} ± 0.00	0.00 ^{b} ± 0.00	0.00 ^{b} ± 0.00	0.00 ^{b} ± 0.00	2.25 *
	Growth inhibition %	0.00 %	88.89 %	100 %	100 %	100 %	100 %	-----

- (*) = Significance at $P \leq 0.05$. - LSD = Least significance difference among means at $P \leq 0.05$.
-Means with different alphabetical superscripts in the same row are significantly different at $P \leq 0.05$).

Table (2). In vitro mean count and percentage of growth inhibition of T. gallinae per culture (trophozoite number x 10^4) after exposure to various concentrations of MTZ in comparison to non treated control (Mean ± SE, n = 4)

Time after treatment in vitro	Mean ± SE and Growth inhibition%	Concentrations of metronidazole (MTZ)						LSD
		None treated control	12.5 µg / ml	25 µg / ml	50 µg / ml	75 µg / ml	100 µg / ml	
24 h	Mean ± SE	9.50 ^{a} ± 0.29	5.75 ^{b} ± 0.48	3.25 ^{c} ± 0.47	0.00 ^{d} ± 0.00	0.00 ^{d} ± 0.00	0.00 ^{d} ± 0.00	2.50 *
	Growth inhibition%	0.00 %	39.47 %	65.79 %	100 %	100 %	100 %	-----
48 h	Mean ± SE	6.50 ^{a} ± 0.29	3.00 ^{b} ± 0.41	0.00 ^{c} ± 0.25	0.00 ^{c} ± 0.00	0.00 ^{c} ± 0.00	0.00 ^{c} ± 0.00	1.25 *
	Growth inhibition%	0.00 %	53.85 %	100 %	100 %	100 %	100 %	-----
72 h	Mean ± SE	2.75 ^{a} ± 0.25	0.00 ^{b} ± 0.00	0.00 ^{b} ± 0.00	0.00 ^{b} ± 0.00	0.00 ^{b} ± 0.00	0.00 ^{b} ± 0.00	2.50 *
	Growth inhibition%	0.00 %	100 %	100 %	100 %	100 %	100 %	-----

- (*) = Significance at P ≤ 0.05. - LSD = Least significance difference among means at P ≤ 0.05.
-Means with different alphabetical superscripts in the same row are significantly different at P ≤ 0.05.

Table (3). Mean count/ High Power Field (HPF) and population density of living *T. gallinae* trophozoites in crop of pigeon squabs during treatment with different concentrations of AGE (Mean ± SE, n = 10)

Group / Days during treatment		Groups and different concentrations					LSD
		Non-infected & Non-treated (Control -Ve) (1)	Experimentally infected with T. gallinae and non-treated (Control +Ve) (2)	Experimentally infected with T. gallinae and treated with AGE 200 mg/ml (3)	Experimentally infected with T. gallinae and treated with AGE 100 mg/ml (4)	Experimentally infected with T. gallinae and treated with AGE 50 mg/ml (5)	
1		0.00 ^{e} ± 0.00 (-)	168.20 ^{a} ± 2.06 (++++)	78.30 ^{c} ± 1.68 (+++)	74.90 ^{c} ± 3.15 (+++)	111.20 ^{b} ± 2.36 (++++)	28.3*
2		0.00 ^{e} ± 0.00 (-)	184.00 ^{a} ± 2.01 (++++)	37.40 ^{d} ± 1.49 (++)	56.70 ^{c} ± 1.61 (+++)	66.40 ^{b} ± 1.50 (+++)	9.4*
3		0.00 ^{e} ± 0.00 (-)	170.90 ^{a} ± 2.97 (++++)	14.50 ^{d} ± 0.60 (++)	35.50 ^{c} ± 0.68 (++)	44.95 ^{b} ± 1.06 (++)	9.4*
4		0.00 ^{d} ± 0.00 (-)	158.80 ^{a} ± 2.20 (++++)	0.50 ^{c} ± 0.17 (+)	13.60 ^{b} ± 0.48 (++)	16.10 ^{b} ± 0.88 (++)	13.1*
5		0.00 ^{c} ± 0.00 (-)	136.20 ^{a} ± 5.66 (++++)	0.00 ^{c} ± 0.00 (-)	3.60 ^{b} ± 0.25 (++)	8.30 ^{b} ± 0.58 (++)	7.1*
6		0.00 ^{c} ± 0.00 (-)	119.90 ^{a} ± 1.50 (++++)	0.00 ^{c} ± 0.00 (-)	0.40 ^{c} ± 0.16 (+++)	3.20 ^{b} ± 0.29 (++)	2.0*
7		0.00 ^{c} ± 0.00 (-)	108.00 ^{a} ± 0.99 (++++)	0.00 ^{c} ± 0.00 (-)	0.00 ^{c} ± 0.00 (-)	0.90 ^{b} ± 0.23 (+)	107.1*
Re-covery	R/T	Not treated	Not treated	5/5	4/5	3/5	-----
	%	-----	-----	100 %	80 %	60 %	-----

- = no living parasite / HPF. + = one living parasite / HPF. ++ = 2-50 living parasite / HPF. +++ = 51-100 living parasite / HPF. ++++ = 101-200 or more living parasite / HPF. - (*) = Significance at P ≤ 0.05. - LSD = Least significance difference among means at P ≤ 0.05. -Means with different alphabetical superscripts in the same row are significantly different at P ≤ 0.05. - R=Responded birds for treatment. - T= Total survivor birds PT.

Table (4). Mean count/High Power Field (HPF) and population density of living *T. gallinae* trophozoites in crop of pigeon squabs during treatment with AGE and MTZ (Mean ± SE, n = 10)

Group / Days during treatment		Non-infected & Non-treated (Control -Ve) (1)	Experimentally infected with T. gallinae and non-treated (Control +Ve) (2)	Experimentally infected with T. gallinae and treated with AGE 200 mg/ml (3)	Experimentally infected with T. gallinae and treated with MTZ (Flagyl®) (4)	LSD
1		0.00 ^{c} ± 0.00 (-)	154.10 ^{a} ± 1.54 (++++)	80.00 ^{b} ± 0.97 (+++)	81.40 ^{b} ± 0.72 (+++)	71.70*
2		0.00 ^{c} ± 0.00 (-)	157.10 ^{a} ± 2.59 (++++)	40.70 ^{b} ± 0.94 (++)	39.70 ^{b} ± 1.19 (++)	39.70*
3		0.00 ^{c} ± 0.00 (-)	166.11^{a} ± 1.80 (++++)	15.30 ^{b} ± 0.56 (++)	15.30 ^{b} ± 0.47 (++)	15.30*
4		0.00 ^{c} ± 0.00 (-)	140.70 ^{a} ± 1.16 (++++)	1.00 ^{b} ± 0.28 (+)	0.90 ^{b} ± 0.32 (+)	139.70*
5		0.00 ^{b} ± 0.00 (-)	124.92 ^{a} ± 1.66 (++++)	0.00 ^{b} ± 0.00 (-)	0.50 ^{b} ± 0.17 (+)	124.40*
6		0.00 ^{b} ± 0.00 (-)	121.70 ^{a} ± 1.35 (++++)	0.00 ^{b} ± 0.00 (-)	0.20 ^{b} ± 0.13 (+)	121.40*
7		0.00 ^{b} ± 0.00 (-)	112.30 ^{a} ± 0.87 (++++)	0.00 ^{b} ± 0.00 (-)	0.00 ^{b} ± 0.00 (-)	121.30*
Recovery	R/T	Not treated	Not treated	10/10	11/11	-----
	%	-----	-----	100 %	100 %	-----

- = no living parasite / HPF. + = one living parasite / HPF. ++ = 2-50 living parasite / HPF. +++ = 51-100 living parasite / HPF. ++++ = 101-200 or more living parasite / HPF. - (*) = Significance at $P \leq 0.05$. - LSD = Least significance difference among means at $P \leq 0.05$. -Means with different alphabetical superscripts in the same row are significantly different at $P \leq 0.05$. - R=Responded birds for treatment. - T= Total survivor birds PT.

Table (5). Some haematological parameters at 2 weeks PT in infected pigeons with *T. gallinae* and treated with AGE and MTZ for seven successive days. (Mean ± SE, n = 5)

Group / Parameter	Non-infected & Non-treated (Control -Ve) (1)	Experimentally infected with T. gallinae and non-treated (Control +Ve) (2)	Experimentally infected with T. gallinae and treated with AGE 200 mg/ml (3)	Experimentally infected with T. gallinae and treated with MTZ (Flagyl®) (4)	LSD
Erythrocytes (RBCs) (cell x10^{6}/ml)	2.924 ^{a} ± 0.015	2.656 ^{c} ± 0.018	2.878 ^{ab} ± 0.014	2.832 ^{b} ± 0.018	0.09*
Haemoglobin (Hb) (gm/dl)	11.332 ^{a} ± 0.054	9.924 ^{c} ± 0.023	10.996 ^{b} ± 0.051	10.802 ^{b} ± 0.481	0.40*
Total Leucocytes (WBCs) (cell x10^{3}/ml	14.874 ^{c} ± 0.036	20.098 ^{a} ± 0.360	15.988 ^{b} ± 0.354	16.540 ^{b} ± 0.258	1.11*
Heterophils (cell x10^{2}/ml)	4.158 ^{c} ± 0.017	5.766 ^{a} ± 0.047	4.984 ^{b} ± 0.414	5.066 ^{b} ± 0.053	0.70*
Lymphocytes (cell x10^{2}/ml)	9.066 ^{d} ± 0.017	12.672 ^{a} ± 0.052	9.426 ^{c} ± 0.056	9.702 ^{b} ± 0.058	0.27*
Monocytes (cell x10^{2}/ml)	0.854 ^{d} ± 0.005	1.202 ^{a} ± 0.009	0.918 ^{c} ± 0.010	0.966 ^{b} ± 0.005	0.05*
Esinophils (cell x10^{2}/ml)	0.364 ^{d} ± 0.005	0.530 ^{a} ± 0.009	0.414 ^{c} ± 0.007	0.472 ^{b} ± 0.014	0.05*
Basophils (cell x10^{2}/ml)	0.348 ^{c} ± 0.005	0.498 ^{a} ± 0.004	0.398 ^{b} ± 0.007	0.404 ^{b} ± 0.008	0.05*

- (*) = Significance at $P \leq 0.05$. - LSD = Least significance difference among means at $P \leq 0.05$.
-Means with different alphabetical superscripts in the same row are significantly different at $P \leq 0.05$.

Table (6). Some boichemical parameters at 2 weeks PT in infected pigeons with *T. gallinae* and treated with AGE or MTZ for seven days. (Mean ± SE, n = 5)

Group / Parameter	Non-infected & Non-treated (Control -Ve) (1)	Experimentally infected with T. gallinae and non-treated (Control +Ve) (2)	Experimentally infected with T. gallinae and treated with AGE 200 mg/ml (3)	Experimentally infected with T. gallinae and treated with MTZ (Flagyl®) (4)	LSD
Serum Total Protein (g/dl)	4.600 ^{a} ± 0.034	3.326 ^{c} ± 0.024	3.946 ^{b} ± 0.049	3.842 ^{b} ± 0.052	0.52*
Serum albumin (g/dl)	2.356 ^{a} ± 0.017	1.634 ^{d} ± 0.024	1.940 ^{b} ± 0.021	1.832 ^{c} ± 0.028	0.11*
Serum globulin (g/dl)	2.240 ^{a} ± 0.043	1.692 ^{c} ± 0.015	2.006 ^{b} ± 0.063	2.010 ^{b} ± 0.050	0.23*
Total cholesterol (mg/dl)	161.968 ^{c} ± 1.101	191.694 ^{a} ± 2.267	162.130 ^{c} ± 1.185	177.754 ^{b} ± 1.961	13.94*
Serum AST (IU/L)	28.192 ^{d} ± 0.036	57.912 ^{a} ± 0.203	35.510 ^{c} ± 0.257	41.206 ^{b} ± 0.740	5.70*
Serum ALT (IU/L)	13.518 ^{d} ± 0.184	36.216 ^{a} ± 0.340	24.108 ^{c} ± 0.384	27.107 ^{b} ± 0.301	3.00*

- (*) = Significance at P ≤ 0.05. - LSD = Least significance difference among means at P ≤ 0.05.
-Means with different alphabetical superscripts in the same row are significantly different at P ≤ 0.05.

Table (7). Changes in body weight (gm) of squabs infected with *T. gallinae* and treated with AGE and MTZ for seven successive days. (Mean ± SE, n = 12)

Group / Days PT	Non-infected and Non-treated (Control -Ve) (1)	Experimentally infected with T. gallinae and non-treated (Control +Ve) (2)	Experimentally infected with T. gallinae and treated with AGE 200 mg/ml (3)	Experimentally infected with T. gallinae and treated with MTZ (Flagyl®) (4)	LSD
0 Day	99.47 ^{a} ± 1.04	80.53 ^{c} ± 1.17	92.73 ^{b} ± 1.57	90.87 ^{b} ± 1.81	7.73*
7 Day	126.40 ^{a} ± 1.62	89.13 ^{c} ± 2.09	121.13 ^{b} ± 2.14	119.47 ^{b} ± 1.80	5.27*
14 Day	163.47 ^{a} ± 2.88	111.47 ^{d} ± 1.55	139.87 ^{b} ± 1.79	125.13 ^{c} ± 2.09	13.67*
21 Day	198.67 ^{b} ± 2.82	141.60 ^{c} ± 2.56	215.87 ^{a} ± 2.15	191.67 ^{b} ± 2.48	17.20*
28 Day	228.40 ^{b} ± 2.78	172.01 ^{d} ± 1.73	245.46 ^{a} ± 3.27	218.33 ^{c} ± 4.57	9.62*

- (*) = Significance at P ≤ 0.05. - LSD = Least significance difference among means at P ≤ 0.05.
-Means with different alphabetical superscripts in the same row are significantly different at P ≤ 0.05.
-0 day = Day of infection. PT= Post treatment.

Table (8). Mortality % of pigeons infected with *T. gallinae* and treated with AGE and MTZ for seven successive days.

Group / Days PT		Non-infected and Non-treated (Control -Ve) (1)	Experimentally infected with *T. gallinae* and non-treated (Control +Ve) (2)	Experimentally infected with *T. gallinae* and treated with AGE 200 mg/ml (3)	Experimentally infected with *T. gallinae* and treated with MTZ (Flagyl®) (4)
No. of pigeons / group		12	12	12	12
No. of dead pigeons	0 Day	0	0	0	0
	7 Day	0	1	0	1
	14 Day	0	2	1	1
	21 Day	0	1	0	0
	28 Day	0	1	0	0
Mortality	Rate	0/12	5/12	1/12	2/12
	%	0.00%	41.67%	8.33%	16.67%

-0 day = Day of infection. PT= Post treatment.

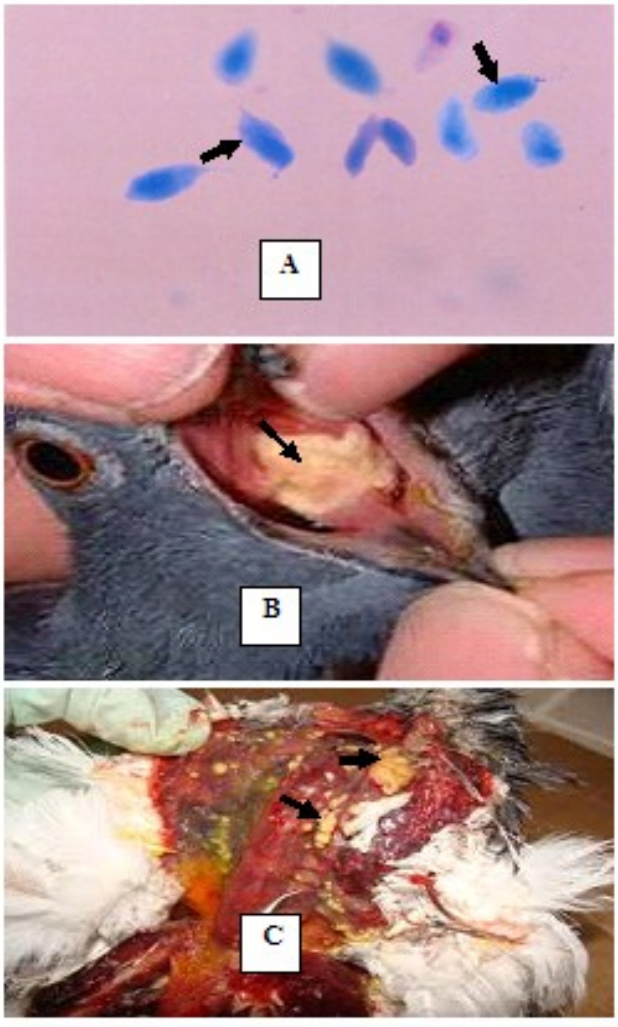

Figure (1).
A- *Trichomonas gallinae* parasite from buccal cavity of naturally infected pigeon squab stained with Geimsa stain (X 1000).
B- Pigeon infected with *Trichomonas gallinae* showing yellowish-white caseous material in the oral cavity.
C- Liver of pigeon infected with *Trichomonas gallinae* showing small yellowish necrotic foci.

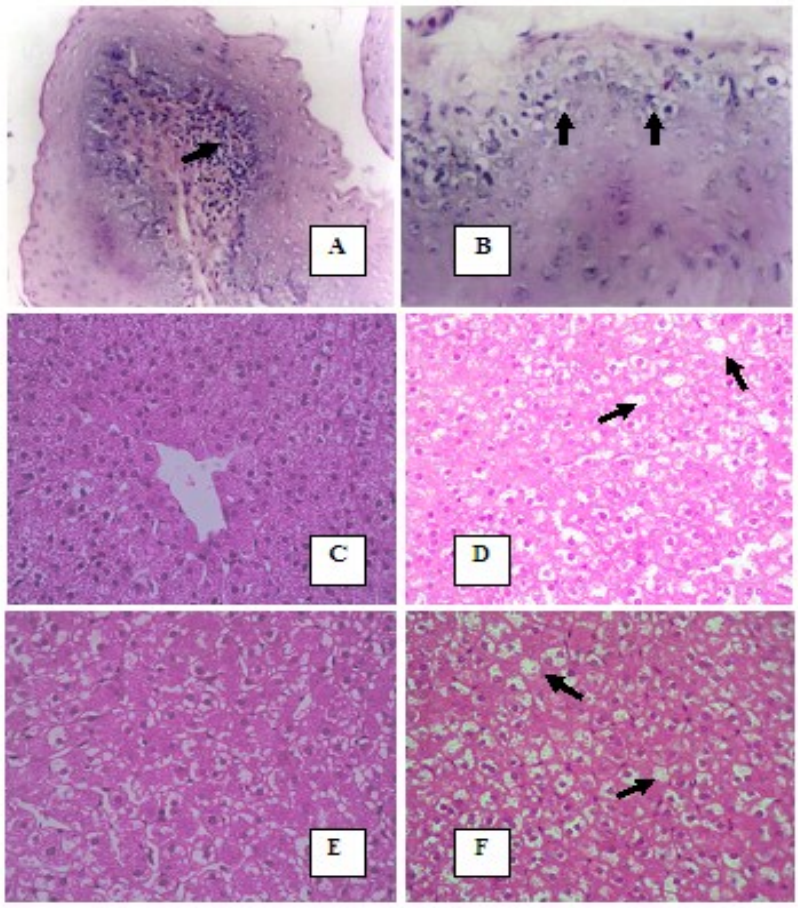

Figure 2:

A- Photograph of crop of squab infected with *T. gallinae* and not treated (positive control) showing necrosis and focal submucosal leucocytic aggregations. (H&E, X 200).

B- Photograph of crop of squab infected with *T. gallinae* and not treated (positive control) showing vacuolation of mucosal epithelium. (H&E, X 400).

C- Photograph of liver of apparently healthy none infected squab (negative control) showing normal histological architectures of hepatocytes. (H&E, X 400).

D- Photograph of liver squab infected with *T. gallinae* and not treated (positive control) showing necrosis and severe hydropic degeneration in hepatocytes. (H&E, X 400).

E- Photograph of liver squab infected with *T. gallinae* and treated with AGE showing mild degenerative changes in hepatocytes. (H&E, X 400).

F- Photograph of liver squab infected with *T. gallinae* and treated with MTZ showing hydropic degeneration in hepatocytes. (H&E, X 400).

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تأثير مستخلص الثوم المائي على طفيل ترايكوموناس جاللينى في الحمام

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الملخص العربى

هدفت هذه الدراسة إلى تأثير المستخلص المائي للثوم على نمو وتكاثرطفيل ترايكوموناس جاللينى بالمزرعة في المعمل وفي العائل مقارنة مع عقار المترونيدازول. قد تم تحضير تركيزات مختلفة من المستخلص المائي للثوم حتى وصلت 200, 100, 75, 50, 25 مجم/مل مذابة في وسط التحضين (GSB). بينما تمت اذابة خمسة تركيزات من المترونيدازول وهي 100, 75, 50, 25, 12,5 ميكروجرام/مل. وتلاحظ نمو الطفيل بعد 24, 48, 72 ساعة عند 37ºم. كما تم تقييم كفاءة الثوم والمترونيدازول مع تحديد اقل تركيز لقتل كل الطفيليات بالمزرعة. قد استخدم 48 زغلول حمام لمقارنة مستخلص الثوم المائي مع الفلاجيل على الطيور. قسمت هذه الطيور الى اربعة مجموعات متساوية كل منها به 12 زغلول، وتركت المجموعة الاولى كضابط سلبي، بينما تم عدوى باقي المجموعات معمليا بجرعة 1x10^{4}من طفيل ترايكوموناس جاللينى عند عمر 21 يوم. تركت المجموعة التانية كضابط موجب، وعولجت المجموعة الثالثة بمستخلص الثوم المائي بجرعة 200مجم/كجم وزن حي، بينما عولجت المجموعة الرابعة بعقار المترونيدازول (فلاجيل شراب) بجرعة 50 مجم/كجم وزن حي. كان العلاج في مياه الشرب ولمدة أسبوع ابتداء من يوم العدوى. تم تحديد عدد طفيليات ترايكوموناس جاللينى من داخل حوصلة الحمام (crop) في كل مجموعة يوميا اثناء فترة العلاج (من اليوم الاول وحتى اليوم السابع) وتم حساب كفاءة الثوم والفلاجيل. تم تحديد مدى إستجابة الطيور للعلاج، وتم اخذ عينات الدم بعد اسبوعين من العلاج لعمل صورة دم كاملة، ولفصل السيرم وذلك لقياس ALT,AST والكوليستيرول الكلي، والبروتين الكلي والالبيومين والجلوبيولين. تم وزن الحمام عند 7, 14, 21, 28 يوم بعد العلاج. تم إجراء بعض الدراسات الباثولوجية على الحوصلة والكبد. اوضحت النتائج بعد عمل مزرعة لطفيل ترايكوموناس جاللينى ان أقل تركيز مثبط لنمو الطفيل نهائيا مع المستخلص المائي للثوم هو 75مجم/مل بعد 24 ساعة، و50مجم/مل بعد 48 ساعة، و25مجم/مل بعد 72ساعة, بالرغم من أن أقل تركيز من المستخلص المائي هو 25مجم/مل فشل في التثبيط النهائي لنمو الطفيل إلا أنه أحدث تثبيط للنمو بما يتراوح من 43,24% الى 88.89 % في كل فترات العلاج بالمزرعة، وقد تم تمت ملاحظة تأثيرات مشابهة مع عقار المترونيدازول حيث كان أقل تركيز مثبط لنمو الطفيل نهائيا هو 50 ميكروجرام/مل بعد 24 ساعة، و25 بعد 48ساعة، و 12 بعد 72ساعة. كما أوضحت النتائج قلة كثافة الطفيل تدريجيا في المجموعة المعالجة حتى إنعدامها تماما بعد اليوم الخامس بعد العلاج بالمستخلص المائي للثوم بعد اليوم السابع بعد العلاج بالفلاجيل وبنسبة إستشفاء 100 %. كما أوضحت الدراسة تحسن في عدد كرات الدم الحمراء ونسبة الهيموجلوبين، ونقص نسبي في عدد كرات الدم البيضاء الكلي والنوعي. كذلك نقص معنوي في مستوى كل من (AST&ALT)في مصل الدم وإقترابه من المعدل الطبيعي في الطيور المعالجة بالمستخلص المائي للثوم عند مقارنته بالفلاجيل. باجراء الفحوص الباثولوجية أوضحت النتائج وجود مادة صفراء متجبنة في تجويف الفم والحوصلة، وكذلك تضخم الكبد مع وجود بقع تنكرزية صفراء على سطح الكبد. ميكروسكوبيا وجد تنكرز وتآكل في الخلايا المبطنة للحوصلة مع وجود خلايا إلتهابية وكذلك وجود تنكرز واضح في خلايا الكبد للحمام المصاب. تحسنت الصورة الباثولوجية بعد العلاج بالمستخلص المائي للثوم مقارنة بالمترونيدازول. نستخلص من هذه الدراسة أن الثوم له تأثير مثبط لنمو طفيل ترايكوموناس جاللينى يكافئ تأثير عقار المترونيدازول بالمزرعة بل وكان افضل منه في الطيور وهذا ينبئ بأن الثوم يمكن إعتباره من النباتات الطبية المرجوة في علاج الاصابة بطفيل ترايكوموناس جاللينى في الحمام.