

Study of the biological effect of *Thymus vulgaris* extracts on spermatogenesis in experimentally infected white mice Balb/c by *Toxoplasma gondii*

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Abstract— The protozoan *Toxoplasma gondii* has a world-wide distribution, It is an obligate intracellular parasite that appears in many species of mammals and birds. In this study, I have evaluated the effect of Alcoholic extract of *Thymus vulgaris* on Testes weights, Body weight, Gonadosomatic index, Daily Sperm Production, Motility and Viability, Testosterone and Fertility between *Toxoplasma gondii* group as compared to the other groups. The white mice Balb/c (n=60) were divided into four groups, control group (n=15), FT group that received tachyzoites of *T. gondii* (n=15), ST group that received tachyzoites of *T. gondii*, plus Alcoholic extract of *Thymus vulgaris* 1 cc per mouse daily by oral gavages method (n=15), and TT group of Alcoholic extract of *Thymus vulgaris* which received Alcoholic extract of *Thymus vulgaris* 1 cc per rat daily by oral gavages method (n=15). Animals were kept in standard condition. In 4week after inducing toxoplasma, 3 cc blood was collected for testosterone, testes tissues of mice in whole groups were removed and then prepared for analysis. Serum total testosterone and sperm parameters were significantly decreased in groups that were infected with *T. gondii*, in comparison to control and Alcoholic extract of *Thymus vulgaris* groups. Testes weights in toxoplasma group significantly decreased in comparison to control group (P<0.05).

Index Terms— Alcoholic extract, *Thymus vulgaris*, *Toxoplasma gondii*, spermatogenesis, sperm Parameters, testosterone.

1 INTRODUCTION

T*oxoplasma Gondii* is a unicellular parasite belonging to the genus *toxoplasma*. It usually doesn't cause diseases in human beings because our immune system prevents this. However, it can cause a disease called toxoplasmosis in humans, if the immune system is weak. An infection with *toxoplasma gondii* can also cause many complications in pregnant women and the fetus might be fatally affected or may suffer serious disease(1).

The prevalence of human infection by *Toxoplasma* varies greatly between countries. Factors that influence infection rates include diet (prevalence is possibly higher where there is a preference for less-cooked meat) and proximity to cats(2).

The rates of positive sero-prevalence in women at child-bearing age between 1990 and 2000 were 58% in Central European countries, 51–72% in several Latin-American countries and 54–77% in West African countries. Low seroprevalence, 4–39%, was reported in southwest Asia, China and Korea as well as in cold climate areas such as Scandinavian countries (11–28%)(3). Although *T. gondii* infection is thought to be detrimental it may have positive effects on some individuals, contrary to the mainstream view. For example *T. gondii* seems to stop brain deterioration to Alzheimer's disease (4). *T. gondii* has also been linked to pre-natal depression, as well as increased anxiety and depression during pregnancies. It has also been linked with mood disturbances in nonpregnant populations,(5) including schizophrenia and suicidal behavior(6).

eral chronic diseases such as cardiovascular diseases, intestinal inflammatory diseases, inflammatory bowel disease, arthritis, diabetes, allergies, multiple sclerosis, Parkinson's and Alzheimer's diseases, and others (7). A Mediterranean aromatic plant, thyme, has been frequently used for those purposes. Extracts from thyme have been used in traditional medicine for the treatment of several respiratory diseases like asthma and bronchitis (8) and for the treatment of other pathologies thanks to several properties such as antiseptic, antispasmodic, antitussive antimicrobial, antifungal, antioxidative, and antiviral (9,10) Thyme oils have also been described as a strong bactericide against Gram-positive and Gram-negative bacteria and also as a bronchospasmolytic(11,12). However, too many little is known about thyme anti-inflammatory activity. For example, it has been reported that thyme oil reduces NO production in J774A.1 murine macrophages(13). The biological activity of medicinal plants strongly depends on their composition. Major bioactive compounds of the extracts from thyme are carvacrol and thymol(14, 15). Thymol exhibits multiple biological activities including anti-inflammatory(16), immunomodulating(17), antioxidant(18), antibacterial(19), antifungal(20), and free radical scavenging properties (21). Carvacrol also possesses antimicrobial, antifungal, and antioxidant activities (22,23,24), as well as antimutagenic and anticarcinogenic effects (25).

Several conditions can interfere with spermatogenesis and reduce sperm quality and production, more factors such as chemotherapy, drug treatment, toxins, infections, air pollutions and insufficient vitamins intake, parasites such as *T.gondii* tachyzoites have harmful effects on spermatogenesis and sperm normal production(26).

Regarding the lack of data about the possible efficacy of *Thymus vulgaris* extract against toxoplasmosis, the efficacy of herbal extract (the alcoholic extracts) of *Thymus vulgaris* against toxoplasmosis in Balb/c mice was evaluated in this study.

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The use of medicinal plants, or extracts from them, has been traditionally practiced worldwide in the prevention and treatment of sev-

2 MATERIALS AND METHODS

2.1 preparation of the alcoholic extracts

The underground green leaves of *Thymus vulgaris* was collected in April, 2012 from AlSamawa city/ alamuthanna province – Iraq. Leaves were cleaned of dust and crushed to prepare the leaves powder.

The melting (15) grams of leaf thyme powder in 200 ml ethanol (80%), the powder put in the tube extraction thumble in Soxhlet extractor and after (7) hours at (60 CO). The mix was filtered using filter paper and then collect the filtration glass bottles and put in an oven at (40 C0) for drying extract and survival of solid material to prepare the concentrations required to test their impact.

The primary concentration of the alcoholic extracts was prepared, which it attended concentrations other for use in subsequent experiment, and by weighting (5) g of dried alcoholic extract and dissolved substance DMSO, as a solvent has no side effect on the parasite or vivo (27). Where it was thawed (5) g of dried alcoholic extract at (10) mL of DMSO and fuller size to (50) ml sterile distilled water filter diameter (0.22) micrometer type Whatman UK, thus the primary concentration of the alcoholic extracts (100)mg/ ml was obtained (28).

2.2 Toxoplasma gondii infection

T.gondii was maintained by passage in mice every 3 days, Tachyzoites were collected from the peritoneal cavity of infected mice and used to inoculate mice. The mice were intraperitoneally injected with 100 Tachyzoites of *T. gondii* in animals house at the Department of Biology in Al-Muthanna University-Iraq(29)

2.3 Experimental animals

Adult albino male mice (n=60) were included in the present study, They were obtained from the National Center for cancer diseases in Baghdad. The mice were (9) weeks old and weighing 25±1 g each, all animals were housed in temperature room (25C0) and fed with a standard diet and water. The daily intake of animal water was monitored at least one week before to start of treatments in order to determine the amount of water needed per experimental animal. And then, the mice were randomly divided into control (n=15) and experimental (n=45) groups. The control group just received 1 cc distilled water daily. Thereafter, the experimental infected mice (n=30) was split two infected *T. gondii* groups, one of this group was *T. gondii* test group (n=15) and other was *T. gondii* group (n=15) which received 1 cc of Alcoholic extract of *Thymus vulgaris* oral stomach tube daily, fourth experimental group (n=15) received 1cc of Alcoholic extract of *Thymus vulgaris* daily(30). This group was Alcoholic extract of *Thymus vulgaris* test group, at the end of the study, the mice were killed by fracture of spinal cord.

2.4 Body weight, testis weight and Gonado-Somatic-Index (GSI)

weight, (at the beginning and the end of the experiment) each of the mice was weighed. After washing the testicles, their weight was measured. The ratio of the weight of both testicles

to the body weight was calculated and the percentage was determined and recorded as GSI.

$$GSI = [\text{Gonad Weight} / \text{Total Tissue Weight}] \times 100(31).$$

2.5 Daily Sperm Production (DSP)

For determining the number of sperms in each gram of the testicle, the number of the sperms was divided to the weight of the testicles and by dividing the result to 4.84, the rate of daily sperm production was calculated(32)

2.6 Motility

For determining the percentage of sperm motility, ductus deferens was extracted from the body and was incubated in normal saline solution in 37°C. Then the ductus deferens was fragmented to extract the sperms and one drop of the solution containing sperms was put in hemocytometric. The sperms were counted in 10 different visual fields, the number of sperms moving forward was determined and the percentage of the moving sperms was calculated.

2.7 Viability

For determining the percentage of viability of the sperms, After putting the left testicles into normal saline and using homogenizer. One drop of sultion and by adding eosine-nicrosine solution, the percentage of the viable sperms, which did not get colored was calculated.

2.8 Testosterone concentration

From each group, their blood was poured into lab tubes and kept in -40°C for 24 h. Then the blood of the mice was centrifuged and taken to the laboratory for determining the serum concentration of testosterone.

2.9 Fertility

From each group three male mice were chosen and each of them was kept with three female mice in a separate cage. After 10 days, the female mice were taken out, anesthetized and the embryos in their uterus were counted. The ovaries were then extracted and washed in normal saline (sodium chloride solution 0.9%). The number of corpus luteum was counted and the fertility rate was determined by dividing the number of embryos to the corpus luteum (33). In the cases that no embryos were found, the above experiment was repeated for three times.

3 STATISTICAL ANALYSIS

Statistical comparisons were made using the ANOVA test for comparison of data in the control group and the experimental group. The results were expressed as mean standard error of means (±SEM), P values less than 0.05 were considered significant.

4 RESULTS AND DISCUSSION

The results in this study for 4 weeks are illustrated in table and figure (1, 2). There was significant difference in Testes

weights, Body weight and Gonadosomatic index between *Toxoplasma gondii* group as compared to the other groups. Daily Sperm Production, Motility and Viability in 1cc Alcoholic extract of *Thymus vulgaris*/ mouse group was significant difference as compared with the control and other experiment groups. Vs these parameters were significantly decreased in *Toxoplasma gondii* group as compared to the other groups and control group. Moreover the Daily Sperm Production, Motility and Viability in 1cc *Toxoplasma gondii* plus 1 cc Alcoholic extract of *Thymus vulgaris* were significantly higher in comparison to the *Toxoplasma gondii* group. Testosterone levels were significantly increased in 1cc Alcoholic extract of *Thymus vulgaris* / mouse group as compared to the other groups and control group. Besides this parameter was significantly decreased in *Toxoplasma gondii* group. Fertility was significant difference between *Toxoplasma gondii* group as compared to the other groups (control and experiment groups).

Parasitic diseases still cause a major challenge to human well-being, particularly in poor populations living in tropical and subtropical climates with low-income economies (34). Some conventional drugs are unaffordable for them and health facilities are also inaccessible. One of the common infections in tropical and subtropical climates is toxoplasmosis caused by *Toxoplasma gondii*. It is one of the most widespread protozoan parasites, chronically infecting approximately 30% of the global human population (35). *T. gondii* causes severe neurological deficits in immunosuppressed patients (such as those with AIDS) and lymphadenopathy in healthy adults. It can cross the placenta (generally in women with no or low antibody levels) and cause congenital infections characterized by intra-cerebral calcifications, chorioretinitis, hydrocephaly or microcephaly, and convulsions (36). Many parasitic infections lead to acute or subclinical, self-limited glomerulopathy during the early phase of immune stimulation, Toxoplasmosis and schistosomiasis, malaria, filariasis, leishmaniasis, trichinosis, echinococcosis and trypanosomiasis cause to glomerular lesions and urinary abnormalities such as proteinuria, lymphocytosis and pyuria were occurred (37). The risks of gestation complications and congenital infections, it has been suggested that toxoplasmosis has some unfavorable effects on reproductive capacity in both men and women (38). The present results clearly indicate that *Thymus vulgaris* has a good effect on spermatogenesis in rats. This results showed that alcoholic extracts of *Thymus vulgaris* for 4 weeks caused a marked increase in testis weight, Body weight, Gonadosomatic index Daily Sperm Production, motility and viability as compared to respective controls. (39) was stressed the plants extracts stimulate appetite in animal and activate the immune response as well as being anti-viruses, bacteria and parasites. Other studies showed the addition of thyme to a bush broiler chicks has led to an improvement in productivity performance and the physiological situation with the non-registration of

any undesirable effects, although the improvement made in this study in body weight because contain thyme on active substances such as thymol and Linalool in addition to other oils (40,41,) and do positive during motivate the functions of the digestive system, liver and pancreas, bile formation and activation for its excretion. As shown (42) a significant increase in the weights of diabetic rats treated with extract and thyme with a low level of glucose, cholesterol, glycerides. This results agree with (43). This effect may be because of contain alcoholic extract of thyme on Glycosides, phenols, terpenes, resins, saponins, tannins, flavonoids, and Coumarin. This is consistent with (44) who pointed out that the extract alcohol contains a high proportion of phenolic compounds characterized by their ability inhibitory against microorganisms, as well as said (45) from the plant contains tannins and terpenes, and alcoholic extract at concentration 70 mg / ml disincentive effect against both bacteria *Pseudomonas aeruginosa* and *Proteus* spp. and *Enterobacter aerogenes*. Came as a result of inhibition of alcoholic extract of thyme to the vitality of the initial approach to the primates reached by researchers from the effect of this extract in a lot of microbiology, and important materials in alcoholic extract is Caffeic acid and Thymol, which constitute 64.8% of the phenolic compounds in thyme (46), which features influencing inhibitory against viruses (43) and against bacteria (47), and against fungi (48), can be attributed to the cause of the ability inhibitory compounds phenolic compounds in the extract alcohol for thyme to the ability of these compounds to inhibit cellular enzymes by oxidizing compounds that have the ability to configure the bonds of hydrogen with effective sites of enzymes for more than a material basis, leading to change their nature.

In the present study, *T. gondii* significantly reduced Daily Sperm Production, motility and viability. In addition, this research showed that the Alcoholic extract of *Thymus vulgaris* can increase the number of sperm and enhanced motility in the group of animals that are infected with *T. gondii*. These results are in agreement with other researchers finding which showed that toxoplasma infection was related to infertility and was possibly related to the antisperm antibodies which can be involved in the pathogenesis of infertility (49). (50) obtained these results in mice study and observed that the acute *T. gondii* infection affects the reproductive function of male mice. *T. gondii* infections have the ability to change the behavior of rats and mice, making them drawn to the scent of cats, rather than being fearful of them. This effect is advantageous to the parasite, which will be able to sexually reproduce if its host is eaten by a cat (51). The infection is highly precise, as it does not affect a rat's other fears such as the fear of open spaces or of unfamiliar smelling food. Studies have also shown behavioral changes in humans, including slower reaction times and a six fold increased risk of traffic accidents among infected males, as well as links to schizophrenia including hallucinations and reckless behavior (52,53).

TABLE 1

The effect of the of 1 cc Alcoholic extract of *Thymus vulgaris* on different parameters male's mice compared with the control group.

Parameters	Control (n=15)	Toxoplasma gondii (n=15)	Toxoplasma gondii plus 1cc Alcoholic extract of <i>Thymus vulgaris</i> (n=15)	1cc Alcoholic extract of <i>Thymus vulgaris</i> / mouse (n=15)
Testes weight (g)	0.1023 ±0.0123	0.0753 ±0.0056 4*	0.1001 ±0.0012	0.0965 ±0.00546
Body weight (g)	24	22.034 ±0.003 4*	23.861 ±0.879	23.323 ±0.23
Gona-dosomatic index (g)	0.5156 ±0.0234	0.3516 ±0.0145*	0.472 ±0.0342	0.502 ±0.0613
Daily Sperm Production (DSP) (%)	47.657 ±3.126*	32.356 ±2.453*	46.54 ±368 ±4.147*	43.56 ±7.798*
Motility(%)	72.100 ±4.367	60.26 ±0.432*	83.37 ±0.434*	72.00 ±4.333*
Viability (%)	69.001 ±4.556	53.86 ±0.546*	68.21 ±0.553	78.35 ±0.754*
Testosterone (ng/ml)	1.546 ±0.234*	0.866 ±0.110*	1.600 ±0.112	2.012 ±0.111*
Fertility (%) Three male from each group	65.54 ±16.345*	34.44 ±0.256*	40.56 ±7.00 ±1.2375*	64.54 ±1.00 ±1.5581*

Mean±SE, N=60. *in each data indicate significant difference at the level of p ≤ 0.05

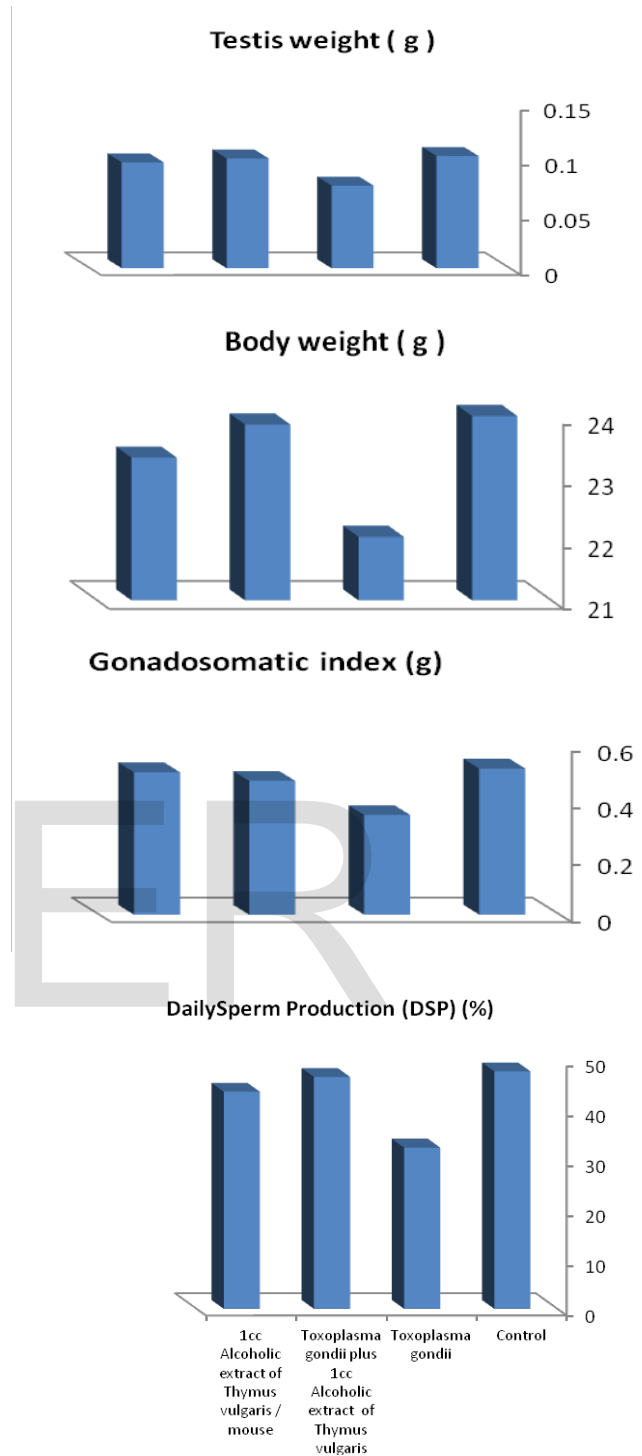


Fig. 1. The effect of the of 1 cc Alcoholic extract of *Thymus vulgaris* on different parameters male's mice compared with the control group.

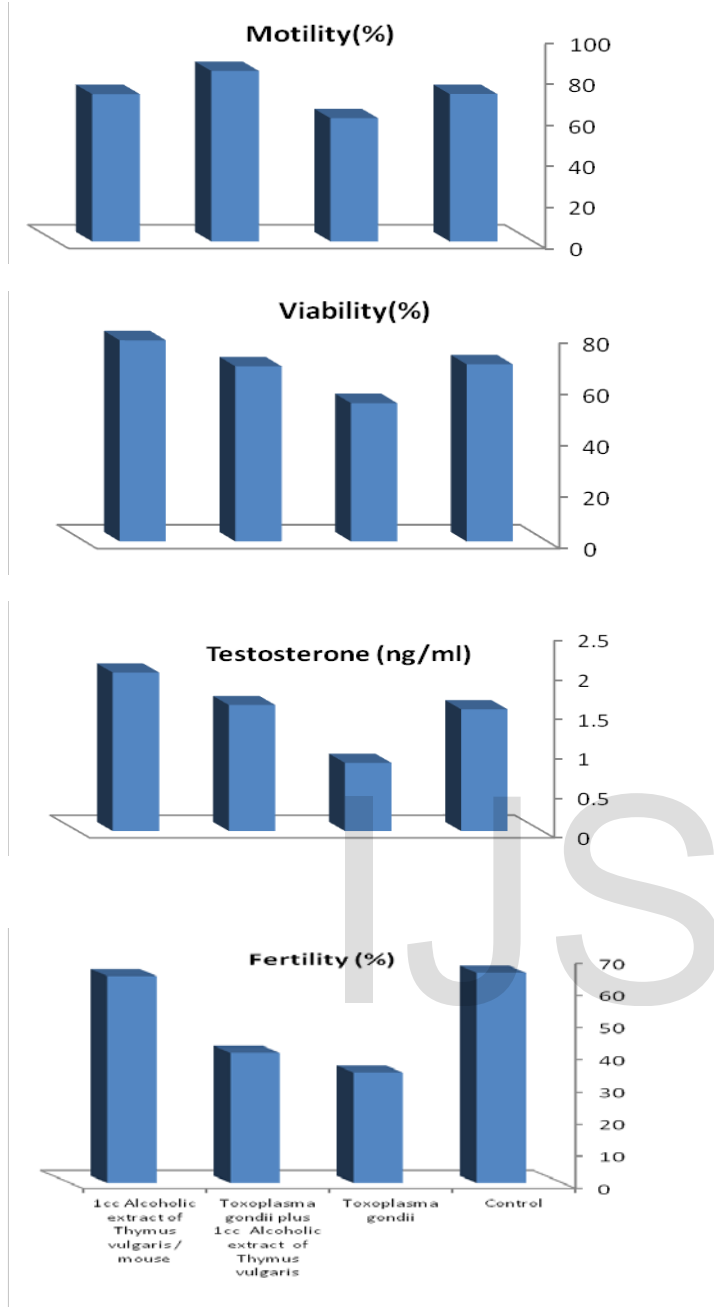


Fig. 2. The effect of the of 1 cc Alcoholic extract of Thymus vulgaris on different parameters male's mice compared with the control group.

5 CONCLUSION

Since in this study, *T. gondii* have significant effect on sperm parameters and serum total testosterone, and since Alcoholic extract of *Thymus vulgaris* was used to treat this harmful effect, it is suggested that eating of *Thymus vulgaris* is useful in infected man.

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