

Full Length Research Paper

Recovery of spermatogenesis by *Allium cepa* in *Toxoplasma gondii* infected rats

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***Toxoplasma gondii* is a protozoan parasite that is globally widespread and infects man and animals; we investigated effect of *Allium cepa* on sperm parameters, testosterone level in male rats experimentally infected by toxoplasma. Wistar male rats (n=40) were allocated into four groups, control group (n=10) and T₁ group that received tachyzoites of *T. gondii* (IP) (n=10), and T₂ group that received tachyzoites of *T. gondii* (IP), plus fresh onion juice 1 cc per rat daily by gavages method (n=10), and group of onion juice which received fresh onion juice 1 cc per rat daily by gavages method (n=10). Animals were kept in standard condition. In 30 day after inducing toxoplasma, 5 cc blood was collected for testosterone and total antioxidant capacity (TAC) levels; testes tissues of rat 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 onions groups. Testes weights in toxoplasma group significantly decreased in comparison to control group (P<0.05). Since in our study, *T. gondii* have significant effect on sperm parameters and serum total testosterone, and since fresh onion juice was used to treat this harmful effect, it is suggested that eating of onion is useful in infected man.**

Key words: *Allium cepa*, testes, testosterone, *Toxoplasma gondii*.

INTRODUCTION

Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii* (Ryan and Ray, 2004). The parasite infects most genera of warm-blooded animals, including humans, but the primary host is the felid (cat) family. Animals are infected by eating infected meat, by ingestion of feces of a cat that has itself recently been infected, or by transmission from mother to fetus. Although cats are often blamed for spreading toxoplasmosis, contact with raw meat is a more significant source of human infections in many countries, and fecal contamination of hands is a greater risk factor (Torda, 2001). Up to one third of the world's human

population is estimated to carry a toxoplasma infection (Montoya and Liesenfeld, 2004). The Centers for Disease Control and Prevention notes that overall seroprevalence in the United States as determined with specimens collected by the National Health and Nutritional Examination Survey (NHANES) between 1999 and 2004 was found to be 10.8%, with seroprevalence among women of childbearing age (15 to 44 years) 11% (Jones et al., 2007). Several conditions can interfere with spermatogenesis and reduce sperm quality and production. More factors such as drug treatment, chemotherapy, toxins, infections, air pollutions and insufficient vitamins intake, parasites such as *T. gondii* tachyzoites have harmful effects on spermatogenesis and sperm normal production (Mosher and Pratt, 1991; Santana et al., 2010). Several studies have reported that antioxidants and vitamin A, B, C and E in diet can protect

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sperm DNA from free radicals and increase blood testis barrier stability (Jedlinska-krakowska et al., 2006). Evidence suggests that *Allium cepa* has antioxidative and androgenic effects in rats and can promote spermatogenesis cycle (khaki et al., 2009). Antioxidants protect DNA and other important molecules from oxidation and damage, and can improve sperm quality and consequently increase fertility rate in men (Yang et al., 2006). Therefore, the role of nutritional and biochemical factors in reproduction and sub-fertility treatment is very important. The present study was planned to assess the ability of *A. cepa* to promote sperm parameters, total antioxidant capacity (TAC) and testosterone concentration, in infected rats by *T. gondii*. The results obtained will provide further insights into appropriate treatment of male infertile patients by using herbs to improve spermatogenesis.

MATERIALS AND METHODS

Plant material

Preparation of onion juice

The underground yellowish-white bulbs of *A. cepa* (onion) was collected in August, 2007 from Ilkhchi in the Province of East Azerbaijan-Iran. The skin was removed and fresh juice of onions was prepared using a Tefal fruit juice extracting machine before the experiments.

Analysis of onion juice

The onion juice was tested for the determination of flavonoids using the Shinoda test (Yousef, 2005). Qualitative thin-layer chromatography (TLC) was employed for determination of quercetin as a main flavonoid in onion. For TLC, 10 ml of fresh onion juice was dried in a vacuum and the resulting residue dissolved in 1 ml of methanol. 20 ml of methanolic solution was spotted on a silica gel plate (10 × 20 cm, silica gel 60 GF254, Merck, Darmstadt, Germany) with a solvent system of EtOAc/MeOH (80:20). Quercetin, Sigma chemical Co. (St. Louis, MO, USA) was used as a control. After developing and drying, the TLC plate was sprayed with 2% AlCl₃ solution in methanol. Quercetin in the onion samples appeared as a yellow spot at RF = 0.6. Separation of quercetin was performed with further purification by preparative TLC on silica gel and quantitative determination of quercetin carried out on a Model 2100 Spectrophotometer (Shimadzu, Japan) in 370 nm compared to a pure quercetin standard curve. The amount of quercetin in fresh onion was 12 mg/100 g.

T. gondii infection

T. gondii strain RH was maintained by passage in mice every 2 days. Tachyzoites were collected from the peritoneal cavity of infected mice and used to inoculate rats. The rats were intraperitoneally injected with 10⁷ tachyzoites of *T. gondii* in house at the Department of Vet pathology in Islamic azad University, Tabriz Branch-Iran (Berdoy et al., 2000).

Experimental animals

Adult Wistar albino male rats (n=40) were included in the present

study. The rats were 8 weeks old and weighing 250±10 g each.

They were obtained from animal facility of Pasture Institute of Iran. Male rats were housed in temperature controlled rooms (25°C) with constant humidity (40 to 70%) and 12/12 h light/ dark cycle prior to experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care[NIH]. All rats were fed with a standard diet and water. The daily intake of animal water was monitored at least one week prior to start of treatments in order to determine the amount of water needed per experimental animal. Thereafter, the rats were randomly divided into control (n=10) and experimental (n=30) groups. The control group just received 4 cc distilled water daily. However, the experimental infected rats (n=20) was split into two infected *T. gondii* groups, one of this group was *T. gondii* test group (n=10) and other was *T. gondii* group (n=10) which received 1 cc of fresh onion juice daily, fourth experimental group (n=10) received 1 cc of fresh onion juice daily (khaki et al., 2009). This group was onion test group, at the end of the study, the rats were killed with carbon dioxide.

Surgical procedure

In thirtieth day, the pentobarbital sodium (40 mg/kg) was administered intra peritoneal for anesthesia, and the peritoneal cavity was opened through a lower transverse abdominal incision. Thereafter testis in control and experimental groups were immediately removed. The weights of testis in each group were registered. The animals were decapitated between 9:00 and 11:00 AM, and blood samples were obtained. Blood samples were centrifuged at 4°C for 10 min at 250 × g and the serum obtained was stored at -20°C until assayed.

Epididymis sperm count, viability and motility

Sperms from the cauda epididymis were released by cutting into 2 ml of medium (Hams F10) containing 0.5% bovine serum albumin (Feng et al., 2001). After 5 min incubation at 37°C (with 5% CO₂), the cauda epididymis sperm reserves were determined using the standard hemocytometric method and sperm motility was analyzed with microscope (Olympus IX70) at 10 field and reported as mean of motile sperm according to WHO (1999) method.

Total antioxidant capacity (TAC) and malondialdehyde (MDA) measurement in serum

A TAC detecting kit was obtained from Nanjing Jiancheng Bioengineering Institute-China. According to this method, the antioxidant defense system, which consists of enzymatic and non-enzymatic antioxidants, is able to reduce Fe³⁺ to Fe²⁺. TAC was measured by the reaction of phenanthroline and Fe²⁺ using a spectrophotometer at 520 nm. At 37°C, a TAC unit is defined as the amount of antioxidants required to make absorbance increase 0.01 in 1 ml of serum (Huang et al., 1995). Free radical damage was determined by specifically measuring MDA. MDA was formed as an end product of lipid peroxidation which was treated with thiobarbituric acid to generate a colored product that was measured at 532 nm (MDA detecting kit from Nanjing Jiancheng Bioengineering Institute-China) (Quintanilha et al., 1982).

Statistical analysis

Statistical comparisons were made using the ANOVA test for comparison of data in the control group and the experimental groups. The results were expressed as mean ± S.E.M (standard

Table 1. The effect of the of 1 cc fresh onion juice /rat on sperm parameters, testosterone, TAC, MDA and testis weight of control and *T. gondii* groups in the rats.

Group	Control	1 ^{cc} fresh onion juice /rat	<i>T. gondii</i>	<i>T. gondii</i> plus,1 ^{cc} fresh onion juice /rat
Testis (g)	1.39 ± 0.55	1.38 ± 0.54	1 ± 0.55*	1.20 ± 0.55
Sperm concentration (total count) (No of sperm/rat × 10 ⁶)	50.11±0.11	61.22 ± 0.33*	40.01 ± 0.55*	44.33 ± 4.43*
Motility (%)	29.65±5.22	64 ± 1.11*	20.12 ± 0.33*	28.32 ± 0.33
Viability(%)	65.15±4.55	74.11 ± 0.56*	50.11 ± 0.55*	63.11 ± 0.55
Total Antioxidant capacity (mmol/ml)	0.60±0.55	1±0.01*	0.50±0.55*	0.60 ± 0.56
Malondialdehyde (mmol/ml)	3.70±0.55	1.22 ± 0.11*	4.90 ± 0.55*	4.1 ± 0.55*
Testosterone (ng/ml)	1.22±0.11	2.46 ± 0.11*	0.87 ± 0.11*	1 ± 0.11

Data are presented as mean ± SE. *Significant different at P< 0.05 level (compared with the control group).

error of means). Significant difference is written in parentheses.

RESULTS

Weight of individual male testis

The obtained results in this study are illustrated in Table 1. There was significant difference in testes weights between *T. gondii* groups as compared to the other groups (p<0.05) (Table 1).

Results of sperm motility, viability and count

Administration of 1 cc fresh onion juice /rat for thirty consecutive days significantly increased sperm counts, motility and viability in onion group as compared with the control and other experiment groups (p<0.05). Besides, these parameters were significantly decreased in *T. gondii* groups (p<0.05) as compared to control and other experiment groups. However, the sperm counts, motility in *T. gondii* group that received 1 cc fresh onion juice /rat were significantly higher in comparison to the *T. gondii* group (Table 1).

Results of total antioxidant capacity and malondialdehyde concentration measurement in serum

The mean concentration of MDA level was not significantly (p>0.05) difference in all groups in comparison to control group. TAC was significantly higher (p<0.05) in fresh onion juice groups as compared with *T. gondii* group (Table 1).

Results of testosterone levels

Levels of testosterone was significantly increased in fresh

onion juice group as compared to control and *T. gondii* groups (p<0.05); this results is higher in infected rats with *T. gondii* that received 1 cc fresh onion juice as compared to *T. gondii* group.

DISCUSSION

T. gondii infection is associated with a wide spectrum of clinical pictures in man. It has been well documented that toxoplasmosis is of crucial importance especially for pregnant women and immunocompromised patients. In addition to 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 (Aral et al., 2011). The data obtained from limited studies performed in animal models as well as in infertile couples, have supported the relationship between toxoplasma and infertility, previous study has indicated toxoplasmosis correlates strongly with an increase in male births in humans (Kanková et al., 2007). According to the researchers, "depending on the antibody concentration, the probability of the birth of a boy can increase up to a value of 0.72 ... which means that for every 260 boys born, 100 girls are born." The study also notes a mean rate of 0.608 (as opposed to the normal 0.51) for toxoplasma-positive mothers. The study explains that this effect may not significantly influence the actual sex ratio of children born in countries with high rates of latent toxoplasmosis infection because "In high-prevalence countries, most women of reproductive age have already been infected for a long time and therefore have only low titres of anti-toxoplasma antibodies. Our results suggest that low-titre women have similar sex ratios to toxoplasma-negative women" (Kanková et al., 2007). It was revealed that INF-c plays an important role in preventing the reactivation of *T. gondii* (Kang and Suzuki, 2001; Wang et al., 2004, 2005, 2007). Non-T cells and

CD8- positive T cells were reported as sources of IFN- γ during chronic *T. gondii* infection which prevents reactivation (Khan et al., 1999; Wang et al., 2005, 2007). It was also reported that IL-12 is required for the maintenance of IFN- γ production of T cells during chronic *T. gondii* infection (Yap et al., 2000). Onion and garlic contain a wide variety of phytochemicals and micro constituents such as trace elements, vitamins, fructans, flavonoids, and sulphur compounds, which may have a protective effect against free radicals. Recently, much attention has been focused on the protective effects of onion against colon cancers in rats (Fukushima et al., 1997; Ross et al., 2006). The present results clearly indicate that *A. cepa* (onion) has a good effect on spermatogenesis in rats. Our results showed that administration of onion juice (1 g/rat/day) for 20 consecutive days caused a marked increase in sperm count, viability, and motility, as compared to respective controls and this agrees with our previous research (Khaki et al., 2009). These effects could be related to vitamins, vitamin C, and flavonoids of onion such as quercetin. Oxidative damage was ascertained by measuring malondialdehyde levels, reactive oxygen species (ROS) generation, alterations in antioxidant defences, and the extent of protein oxidation. Quercetin, an important flavonoid, has a beneficial effect on health due to its antioxidant function. Studies on the effect of quercetin on oxidative damage in cultured chicken spermatogonial cells showed quercetin to have no deleterious effect on spermatogonial cells at doses of 1 and 10 mg/ml. Quercetin (1 mg/ml) increased the number of spermatogonial cells and decreased the mortality of aroclor-induced oxidative damage. In this study, the effect of quercetin on serum MDA was determined, but the results indicated no obvious effect of quercetin on MDA production (Mi and Zhang, 2005; Mi et al., 2007). In the present study, *T. gondii* significantly reduced sperm count and motility. On the other hand, our researches showed that the fresh juice of onion can increase the number of sperm and enhanced motility ability, 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 (Zhou et al., 2002; Aral et al., 2011). Sun et al. (2008) 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 (Berdoy et al., 2000).

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 (Flegr et al., 2002), as well as links to schizophrenia including hallucinations and reckless behavior.

Antioxidants and vitamins from foods consumed by animals, such as quercetin, vitamin C, vitamin B and vitamin E, could improve sperm health parameters and testicular androgenesis. In our research, the results showed that onion fresh juice could significantly increase the recovery of sperm health parameters, such as count, motility and serum total testosterone and TAC levels in infected rats. In another study that was done by McCarthy et al. (2003), they showed that vitamin E and selenium could result in trends toward increased tissue cyst number, tissue pathology, and weight loss during infection.

Conclusion

In our study, *T. gondii* have significant effect on sperm parameters and serum total testosterone. On the other hand, freshly prepared onion juice significantly affected the sperm number, percentage of viability and motility, and was used for treating this harmful effect. So, it is suggested that eating of onion is useful in infected men.

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