INTERNATIONAL JOURNAL OF WOMEN'S HEALTH AND REPRODUCTION SCIENCES
http://www.ijwhr.net
doi: 10.15296/ijwhr.2014.12

Repairing Effect of Allium Cepa on Testis Degeneration Caused by Toxoplasma Gondii in The Rat

Yagoob Garedaghi[1*] , Seyed Razi Bahavarnia [2]

Article History:
Received 9 October 2013
Accepted 7 January 2014
Revised 25 December 2013
Available online 7 January 2014

Keywords:
Allium Cepa
Testis Degeneration
Toxoplasma Gondii

Corresponding Author:
Yagoob Gharedaghi, Assistant professor, PhD, Department of Veterinary Parasitology, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
Tel: +989143110499
Email: y_garedaghi@yahoo.com

Abstract

Objectives: Toxoplasma gondii, an intracellular parasite, infectS a large proportion of the world population yearly. This study was investigated to evaluate the remedial effects of allium cepa on testis degeneration in male rats infected by T.gondii, RH strain.

Materials and Methods: Wistar male rats (n=40) divided into control (n=10) and experimental (n=30) groups. The experimental groups were divided into two groups; allium cepa group (n=10) received 1cc of fresh onion juice daily and the toxoplasmose infected group (n =20) were subdivided into two groups of 10. One of the toxoplasma groups also received 1cc of fresh onion juice daily; however, control group just received distilled water. Animals were kept in a standard condition. On day 30 after inducing Toxoplasmosis infection, 5 ml blood sample of each rat was taken to measure serum protein and total antioxidant capacity (TAC) levels. IgG and IgM were tested by the ELISA method. Testicular tissue of each Rat was removed and sperms were collected from the epididymis for analysis.

Results: Serum proteins and testis weight were significantly decreased in the T.gondii groups compared with the control and onion groups. Testis degeneration significantly increased in toxoplasmosis group compared with the control group ($P<0.05$). TAC level was significantly increased in the groups that received onion juice ($P<0.05$).

Conclusion: This study showed T. gondii has diverse effects on serum proteins, TAC, and testis. Results confirmed fresh onion juice could significantly modify harmful effects and increase the sperm number, viability, and motility so it seems eating onion is useful in toxoplasmosis infection.

1 - Assistant professor, PhD, Department of Veterinary Parasitology, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
2- Department of Parasitology, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

Introduction:

Toxoplasma gondii is an intracellular parasite that infects a wide variety of hosts, including humans (1). Human infection occurs through two main routes, ingestion of undercooked meat containing cysts of the parasite and ingestion of oocysts passed into the environment by cats (2). The parasite infects plenty generations of warm-blooded animals, including humans, but the definitive host is the felid (cat) family. Animals are infected by eating infected meat, by ingestion of feces of a cat that has 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 (2). Up to one third of the world human population is estimated to carry a Toxoplasma infection (3). The outcome of toxoplasmosis in the mouse model is strongly dependent on the strain of T. gondii and can be predicted based on the parasite genotype (6, 7). Many parasites such as Cryptosporidium , Toxoplasma , Leishmania, Trypanosoma, Strongyloides, Malaria, and schistosoma cause severe infection in immunocompromised patients. (e.g. toxoplasmosis, and malaria) or a dormant infection may be activated as a result of immunosuppression (e.g. cryptosporidiosis and strongyloidiasis). Several studies have reported that antioxidants and vitamins A, B, C, and E in diet can protect mammalian cells DNA from free radicals (5). Evidences suggest that Allium cepa has antioxidant effects on rats and human (6). Antioxidants protect DNA and other important molecules from oxidation and damage (7). Therefore, the role of nutritional and biochemical factors in immune systems is very important. The present study was planned to assess the ability of Allium cepa for decreased testis degeneration rate in spermatogenic cells in rats infected by Toxoplasma gondii.

Material & Methods:

Plant material

Preparation of onion juice:

The underground yellowish-white bulbs of Allium cepa (onion) was collected from Ilkhchi in the province of East Azerbaijan-Iran. The skin was removed and fresh juice of onions was prepared by using a Tefal fruit juice extracting machine before the experiment.

Analysis of onion juice:

The onion juice was tested for the determination of flavonoids by using the Shinoda test (8). 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 was dissolved in 1ml 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 a 2% AlCl3 solution in methanol. Quercetin in the onion samples was 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 was carried out on a Model 2100 Spectrophotometer (Shimadzu, Japan) in 370 nm comparing to a pure quercetin standard curve. The amount of quercetin in fresh onion was 12 mg/100 g.

T. gondii infection:

Tachyzoites of T. gondii RH strain was maintained by passage in mice every 3 days. Tachyzoites were collected from the peritoneal cavity of infected mice and used to inoculate to the rats. The rats were intraperitoneally injected with 107 tachyzoites of T. gondii (9) in animal house at the Department of Vet pathology in Islamic Azad University, Tabriz Branch-Iran.

Experimental animals:

Adult Wistar albino male rats (n=40) were included in the present study. The rats were 8 weeks old and weighing 250±10g 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 (40-70%)

and 12h/12h 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 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 4CC distilled water daily. The experimental groups were divided into two groups, allium cepa group (n=10) just received 1cc of fresh onion juice daily and the toxoplasmose infected group (n =20) were subdivided into two groups of 10. One of the toxoplasmose groups also received 1cc of fresh onion juice daily (6). 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 testes in control and experimental groups were immediately removed. The weights of testes in each group were registered. The animals were decapitated between 9:00 AM and 11:00 AM, and blood samples were obtained. Blood samples were centrifuged at 4°C for 10 min at 250×g and the obtained sera were stored at −20°C until used.

Epididymal sperm motility, viability and count, and sperm abnormality:

The cauda epididymis was cut and Sperm were released into 2 mL of medium (Hams F10) containing 0.5% bovine serum albumin (15). After 5 min incubation at 37C (with 5% CO2), the cauda epididymis sperm reserves were determined by using the standard haemocytometric method, and sperm motility was analyzed by microscope (Olympus IX70) at 10 field and reported as the mean of motile sperm
according to WHO methods (33). The sperm abnormality was evaluated according to the standard method of Narayana (27). Briefly, smears of the sperm suspension were made on clean glass slides and stained with periodic acid Schiff's reaction haematoxylin. The stained smears were observed under a light microscopic with 40× magnification. The sperms were classified into normal and abnormal. The total sperm abnormality was expressed as percentage incidence.

Histology:

The testis were fixed in 10% formalin and embedded in paraffin. Five-micron-thick sections were prepared and stained with haematoxylin and eosin (HE). The specimens were examined under an Olympus/ 3H light microscope. The diameter of the seminiferous tubules was measured in 20 round tubular sections per animal at 100× magnification and the digitized images were analyzed for morphometric
study. Then D — mean diameter, a — high diameter, and b — low diameter were measured and substituted (as a and b) in this formula: D = ÷a × b. The software for the measurement of the diameters of seminiferous tubules was Image Toll 2007 (15).

Total antioxidant capacity (TAC) 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+ by 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 (11).

Statistical analysis:

Statistical comparisons were made by using the ANOVA test for comparison of data in the control and experimental groups. The results were expressed as mean ± S.E.M (standard error of means). Significant difference was written in parentheses, P< 0.05 level (compared with the control group).

Results:

Weight of individual male testis:

There was no significant change in testis weight between the control and experimental groups (Table 1).

Sperm motility, viability, count, and abnormality:

Administration of 0.5 g/rat and 1 g/rat of fresh onion juice for 30 consecutive days significantly increased sperm motility and viability in both experimental groups as compared to the control group, using the Dunnett homogeneity test (Table 1).

Sperm count was significantly increased in the experimental group that received 1 g/kg freshly prepared onion juice as compared with the control group (Table 1). Sperm abnormality was not significantly different in the experimental group that received 1 g/kg fresh onion juice compared with the control group (Table 1).

Serum analysis for biochemical and serological studies:

Level of Creatinine, total protein and albumin were measured by using kits (Merck Diagnostic Ltd, India) followed by spectrometric methods. The values were expressed in mg dL−1 in all the cases(Table 1). Blood sampling was performed without anticoagulant according to standard techniques and after 30 min, the tubes were centrifuged at 2,000 rpm for 5 min and then sera were aliquoted in several labeled vials and kept frozen at -20<C. For determination of anti-T. gondii antibodies, all sera were tested by Enzyme-Linked Immunosorbent Assay (ELISA) kits. For determination of anti-T. gondii IgG and IgM antibodies of ELISA kitspurchased from the Biotech Trinity Captia TM Company (American) were used. The optical density of IgG antibody titers were read at 490 nm by using automatic ELISA reader 17/4 IU/mL or above were considered positive for T.gondii immunoglobulin G antibodies.

Serum total antioxidant capacity and malondialdehyde concentration:

Administration of 0.5 g/rat and 1 g/rat of fresh onion juice daily for 30 consecutive days significantly decreased the level of MDA concentration in the experimental groups compared to the control group ($p < 0.05$). Administration of 1 g/rat of fresh onion juice daily for 30 consecutive days could significantly increase the level of TAC. However, 0.5 g/rat of fresh onion juice did not have any significant effect on TAC in the experimental groups (Table 1).

Histology:

The histopathological study showed the cycle of spermatogenesis was regular in all experimental and control groups (Fig. 1A), but there was no significant difference in seminiferous tubules between the control group and the group that received 0.5 g/rat of fresh onion juice (Fig. 1B). However, in all animals exposed to 1 g/rat of fresh onion juice, an accumulation of sperm in the lumen of the seminiferous tubules was observed (Fig. 1C). The diameter of the seminiferous tubules showed no significant difference in any animals exposed to 0.5 g/rat and 1 g/rat of fresh onion juice, as compared with those measured in the control group (Table 1).

Discussion:

This study showed that T. gondii has significant effect on serum protein, TAC , IgG, IgM and testis degeneration and fresh onion juice modify these harmful effects. Furthermore, freshly prepared onion juice significantly affected the sperm number, percentage of viability, and motility. Toxoplasma gondii infection is associated with a wide spectrum of clinical manifestations in men, It has been well documented that toxoplasmosis has 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 (12). Researchers revealed that INF-c plays an important role in preventing the reactivation of T. gondii (15,16,17,18) Non-T cells and CD8-positive T cells were reported as sources of IFN-c during chronic toxoplasma infection which prevent reactivation of toxoplasmosis infection (17,18,19). It was also reported that IL-12 is required for the maintenance of IFN-c production of T cells during chronic toxoplasma infection (20).

Toxoplasmosis, an opportunistic protozoan parasite infection, is widespread in humans and animals and emerges as a life-threatening risk in immunocompromi sed individuals (18) . Seroepidemiological survey in different parts of the world indicates that the prevalence rates range

from zero to 98% (8). Testicular degeneration characterized by vacuolar and necrosis of the spermatogonia and spermatocytes with complete arrest of spermatogenesis were found in toxoplasmosis infection. In the testis of animals that exposed for 14 days to Toxoplasma gondii, marked and severe alterations were noticed (5). Following Toxoplasma gondii infection, the Leydig cells and intertubular connective tissue show signs of hypoplasia and the intertubular space become wider. Most of the seminiferous tubules have an irregular shape and the boundary tissue is thinner and breaks in its continuity are observed at multiple locations(3). Damage in the epithelium germinal layer, maturation arrest in the spermatogenesis, and disorder in the germinal cell distribution are recorded. Moreover, atrophied seminiferous tubules decrease in the germ cell population and fusion between some seminiferous tubules is observed (8).

Infertility is one of the major problems in couples' lives; about 25% and 35% of infertility is attributed to the male and the female receptivity, respectively (3). Many environmental and biochemical factors are involved in male and female reproduction (26). The importance of many of these factors has not yet been clearly understood. A better understanding of the underlying mechanisms in subfertility and more studies results clarify the effectiveness of nutritional and biochemical factors in improvement of diagnosis and treatment infertility. Smart choices with regard to a better diet might protect the body from many diseases (5). The main advice for a healthy diet is to eat more fruit and vegetables. However, published interventional attempts have not yet supported this message (2, 31). 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 (10, 29). The present results clearly indicate that Allium 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. These effects could be related to vitamins, vitamin C, and flavonoids of onion such as quercetin.

Oxidative damage is ascertained by measuring malondialdehyde (MDA) 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. One mechanism of the antioxidant action of quercetin is involved in scavenging free radicals such as superoxide

radicals generated by xanthine/xanthine oxidase (7). 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 mg/mL and 10 mg/mL. Khaki et al. reported quercetin (1 mg/mL) increased the number of spermatogonial cells and decreased the mortality of Aroclor-induced oxidative damage(24, 25). In this study, the effect of quercetin on serum MDA was determined, but the results indicated no obvious effect of quercetin on MDA production. Vitamins C and E are well known as antioxidants that can ameliorate oxidative stress-related testicular impairments in animal tissue (8, 11, 16, 21). Vitamin C may execute its role by modulating testicular free radical production and/or stimulating testicular androgenesis and is essential for testicular differentiation, integrity, and steroidogenic functions (6, 19, 30, 32). Furthermore, vitamin C is an antioxidant in semen and thus protects sperm from oxidative damage (4, 34, 35).

The percentage of sperm viability and motility significantly increased in the test groups ($p < 0.05$), but the sperm concentration significantly increased only in the group that received the high dose of fresh onion juice ($p < 0.05$). Moreover, it had no effect on sperm morphology and testis weight in both groups compared to the control group. Thus, it seems that using

1 g/kg of fresh onion juice can be effective for sperm health parameters. The results revealed that MDA decreased but TAC, serum albumine and Total protein increased with onion juice. These alterations could be due to the vitamins and quercetin content of onion. Therefore, these results indicated that antioxidants and vitamins of foods consumed by animals, such as quercetin, vitamin C, vitamin B, and vitamin E, could improve sperm health parameters .

Conclusion :

This study showed that T. gondii has significant diverse effect on serum protein and TAC , IgG and IgM and testes degeneration and fresh onion juice returned and treated these harmful effects. Furthermore, freshly prepared onion juice significantly affected the sperm number, percentage of viability, and motility; it seems that using 4g/kg of freshly prepared onion juice is effective in sperm health parameters, (Folia Morphol 2009; 68, 1: 45–51) so it is suggested that eating of onion is useful in toxoplasma infection.

Conflicts of interest:

Authors declare that there is no any conflict of interest.

Acknowledgments:

The authors would like to thanks Tabriz Branch, Islamic Azad University for the financial supports of this research ,which is based on a research project contract. The code of this research protocol was 7582.

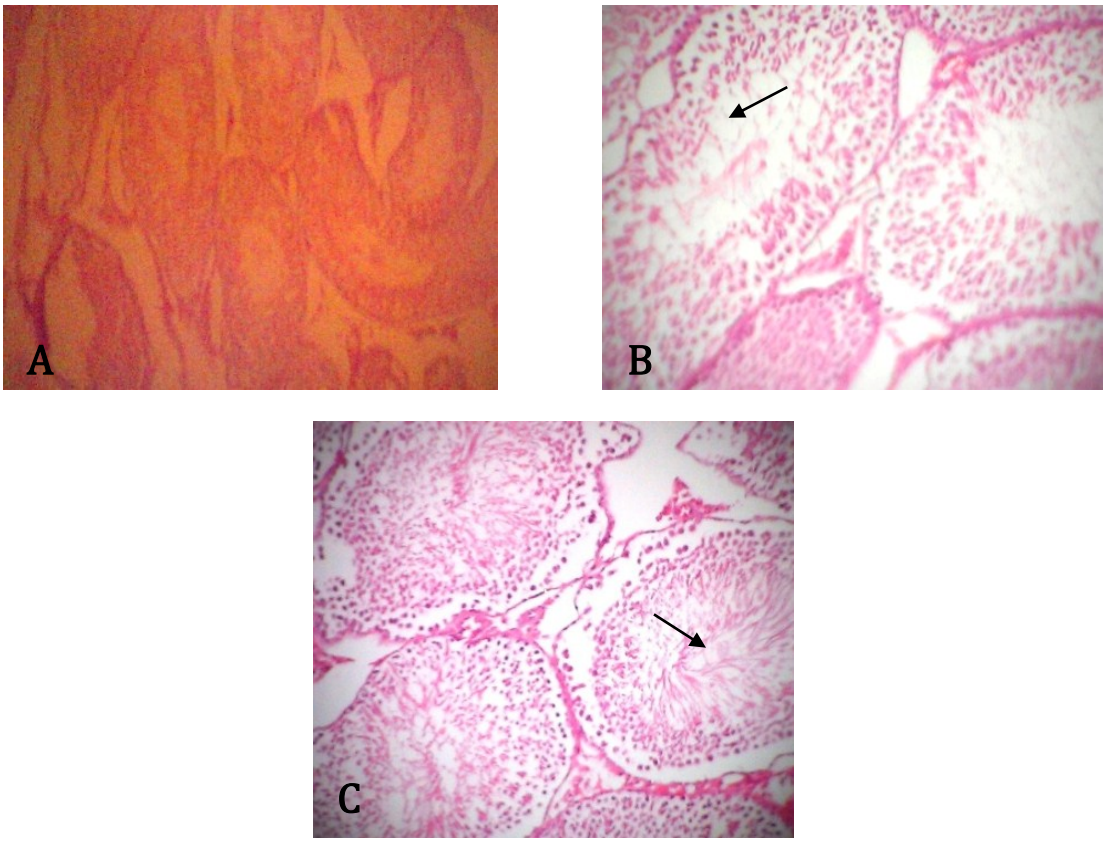
Table 1. The effect of the of 1cc fresh onion juice /rat on sperm parameters, testis weight, and diameter of seminiferous tubules of control and toxoplasma groups in the rats.

Groups	Control	1cc fresh onion juice /rat	Toxoplasma	Toxoplsma plus,1cc fresh onion juice /rat
Testis [g]	1.40 ± 0.832	1.42 ± 0.829	1.26 ± 0.345	1.41 ± 0.834
Serum protein	9±0.4	7.8±0.4	4±0.3	7.9±0.1
Total Antioxidant capacity, (mmol/ml)	0.80±0.45	1±0.02*	0.50±0.53*	0.50±0.45*
Malondialdehyde (MDA)	4.80 ± 0.212	2.51 ± 0.193*	5.33 ± 0.311	3.42 ± 0.358*
Sperm concentration (total count) (No. of sperm/rat × 106)	47.61 ± 7.80	76.70 ± 2.44*	31.64 ± 5.73	59.35 ± 5.48
Motility (%)	33.75 ± 6.88	91 ± 5.23*	21.73 ± 6.66	77 ± 8.82*
Viability (%)	66.25 ± 4.73	70.21 ± 1.30*	51.23 ± 3.63	91.64 ± 1.51*
Diameter of seminiferous tubules [µm]	380.1 ± 0.1	387.5 ± 0.3	359.1 ± 0.2	379.3 ± 0.4
Serum albumin(g/dl)	2.8±0.05	3.4±0.05	2.1±0.05	3.1±0.05
Serum creatinine (mg/dl)	0.1±0.04	0.97±0.21	0.2±0.35	0.17±0.23

Data are presented as mean ± SE.

*Significant different at P< 0.05 level , (compared with the control group).

Figure 1. A. Regular seminiferous tubule with normal germinal epithelium morphology in the control group (H&E, ×320); **B.** Regular seminiferous tubule with normal germinal epithelium morphology and sperm presence. In lumen of seminiferous tubule (arrow) in 0.5 g/rat fresh onion juice group (H&E, ×640); **C.** Regular seminiferous tubule with normal germinal epithelium morphology and sperm presence. In lumen of seminiferous tubule (arrow) in 1 g/rat fresh onion juice group (H&E, ×640).



References:

1. Ryan KJ, Ray CG. Sherris Medical Microbiology(4th ed.). McGraw Hill. 2004;723-7.

2. Torda A.Toxoplasmosis. Are cats really the source?. Aust. Fam. Physician. 2001;30(8): 743-7.

3. Montoya J, Liesenfeld O.Toxoplasmosis. Lancet. 2004; 363 (9425): 1965-76.

4. Jones JL, Kruszon-Moran D, Sanders-Lewis K, Wilson M. Toxoplasma gondii infection in the United States, 1999-2004, decline from the prior decade. Am. J. Trop. Med. Hyg. 2007;77 (3): 405-10.

5. Jedlinska-Krakowska M, Bomba G, Jakubowski K, Rotkiewicz T, Jana B, Penkowski A. Impact of oxidative stress and supplementation with vitamins E and C on testes morphology in rats. J. Reprod. Dev. 2006;52: 203-209.

6. Khaki A, Fathiazad F, Nouri M, Khaki AA, Khamenehi HJ, Hamadeh M. Evaluation of androgenic activity of allium cepa on spermatogenesis in the rat. Folia Morphol. (Warsz). 2009;68(1):45-51.

7. Yang HS, Han DK, Kim JR, Sim JC. Effects of alpha- tocopherol on cadmium-induced toxicity in rat testis and spermatogenesis. J. Korean Med. Sci. 2006;21: 445-451.

8. Yousef MI . Protective effect of ascorbic acid to enhance reproductive performance of male rabbits treated with stannous chloride. Toxicology. 2005;207: 81-89.

9. Berdoy M, Webster JP, Macdonald DW. Fatal attraction in rats infected with Toxoplasma gondii. Proc. Biol. Sci. 2000;267 (1452): 1591-4.

10. Khaki A, Heidari M, Ghaffari Novin M, Khaki AA. Adverse effects of ciprofloxacin on testis apoptosis and sperm parameters in rats. Iranian J. Reprod. Med. 2008;6(2): 71-76.

11. Huang HFS, Linsenmeyer TA, Li MT, Giglio W, Anesetti R, von Hagen J, Ottenweller JE, Pogach L. Acute effects of spinal cord injury on the pituitary-testicular hormone axis and Sertoli cell functions: a time course study. J. Androl. 1995;16: 148-157.

12. Aral-Akarsu G, Elhan HA, Akarsu C. Retrospective evaluation of Toxoplasma gondii seropositivity in fertile and infertile women. Mikrobiyol. Bul. 2011;45(1):174-80.

13. Sitprija V, Benyajati C. Tropical disease and acute renal failure. Ann. Acad. Med. Singapore. 1975; 4:112-124.

14. Barsoum R.S. Tropical parasitic nephropathies. Nephrol. Dial. Transplant. 1999;14(3):79-91.

15. Kang H, Suzuki Y. Requirement of non-T cells that produce gamma interferon for prevention of reactivation of Toxoplasma gondii infection in the brain. Infect Immun. 2001; 69(5):2920-7.

16. Wang X, Kang H, Kikuchi T, Suzuki Y. Gamma interferon production, but not perforin-mediated cytolytic activity, of T cells is required for prevention of toxoplasmic encephalitis in BALB/c mice genetically resistant to the disease. Infect Immun. 2004;72(8):4432-8.

17. Wang X, Claflin J, Kang H, Suzuki Y. Importance of CD8(+)Vbeta8(+) T cells in IFN-gamma-mediated prevention of toxoplasmic encephalitis in genetically resistant BALB/c mice. J. Interferon Cytokine Res. 2005; 25(6):338-44.

18. Wang X, Michie SA, Xu B, Suzuki Y. Importance of IFN-gamma-mediated expression of endothelial VCAM-1 on recruitment of CD8+ T cells into the brain during chronic infection with Toxoplasma gondii. J. Interferon Cytokine Res. 2007;27(4):329-38.

19. Khan IA, Green WR, Kasper LH, Green KA, Schwartzman JD. Immune CD8(+) T cells prevent reactivation of Toxoplasma gondii infection in the immunocompromised host. Infect Immun. 1999;67(11):5869-76.

20. Yap G, Pesin M, Sher A. Cutting edge: IL-12 is required for the maintenance of IFN-gamma production in T cells mediating chronic resistance to the intracellular pathogen, Toxoplasma gondii. J. Immunol. 2000;165(2):628-31.

21. Fukushima S, Takada N, Hori T, Wanibuchi H. Cancer prevention by organosulfur compounds from garlic and onion. J. Cell Bioch. 1997; 27: 100-105.

22. Ross SA, Finley JW, Milner JA. Allyl sulfur compounds from garlic modulate aberrant crypt formation . J. Nutr. 2006;136: 852S-854S.

23. Haidari F, Rashidi MR, Keshavarz SA, Mahboob SA, Eshraghian MR, Shahi MM.Effects of onion on serum uric acid levels and hepatic xanthine dehydrogenase/xanthine oxidase activities in hyperuricemic rats. Pak. J. Biol. Sci. 2008;11(14):1779-84.

24. Khaki A, Farzadi L, Ahmadi Sh, Ghadamkheir E, Khaki AA, Shojaee S, Sahizade R. Recovery of spermatogenesis by Allium Cepa in Toxoplasma gondii infected rats. AJPP. 2011;5(7):903-907.

25. Khaki A, Fathiazad F, Nouri M, Khaki AA, A-Maleki N, Jabbari-Khamnei H, Ahmadi P. Beneficial effects of quercetin on sperm parameters in streptozotocin-induced diabetic male rats. Phytother. Res. 2010;24(9):1285-1291.

26. Mi Y, Zhang C. Protective effect of quercetin on Aroclor 1254-induced oxidative damage in cultured chicken spermatogonial cells. Toxicol. Scien. 2005;88: 545-550.

27. Mi Y, Zhang C, Taya K . Quercetin protects spermatogonial cells from 2,4-d-induced oxidative damage in embryonic chickens. J. Reprod. Dev. 2007;53: 749-754.

28. Rajeev K, Gagan G, Narmada P . Drug therapy for idiopathic male infertility: rationale versus evidence. J Urology.2006; 176: 1307-1312.

29. Ross SA, Finley JW, Milner JA .Allyl sulfur compounds from garlic modulate aberrant crypt formation. J Nutr.2006; 136: 852S-854S.

30. Salem MH, Kamel KI, Yousef MI, Hassan GA, EL-Nouty FD . Protective role of ascorbic acid to enhance semen quality of rabbits treated with sublethal doses of aflatoxin B1. Toxicology.2001; 162: 209-218.

31. Schatzkin A, Lanza E, Corle D . Lack of effect of a low-fat, high-fiber diet on the recurrence of colorectal adenomas. New Engl J Med.2000; 342: 1149-1155.

32. Thews O, Lambert C, Kelleher DK, Biesalski HK, Vaupel P, Frank J . Possible protective effects of alpha-tochopherol on enhanced induction of reactive oxygen species by 2-methoxyestradiol in tumors. Adv Exp Med Biol.2005; 566: 349-355.

33. World Health Organization (1999) WHO Laboratory manual for the examination of human semen and semen- cervical mucus interaction. 4th Ed. Cambridge University Press, New York.

34. Yousef MI, Abdallah GA, Kamel KI . Effect of ascorbic acid and vitamin E supplementation on semen quality and biochemical parameters of male rabbits. Anim Reprod Sci.2003; 76: 99-111.

35. Yousef MI . Protective effect of ascorbic acid to enhance reproductive performance of male rabbits treated with stannous chloride. Toxicology.2005; 207: 81-89.