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Modulating effect of Allium cepa on kidney apoptosis caused by Toxoplasma gondii

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Research Article

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Abstract

Aim: *Toxoplasma gondii* is a widespread protozoan parasite that infects a broad range of warm blooded animals as well as humans. The present study was investigated to evaluate the effects of allium cepa on renal failure in male rats which experimentally infected by *Toxoplasma gondii,* RH strain.
Methods: Wistar male rat (n=40) were allocated into four groups, group one that received tachyzoites of *T. gondii* (ip) (n=10), group two that received tachyzoites of *T. gondii* (ip) , plus fresh onion juice by gavages method (n=10) , group three received just fresh onion juice by gavages method (n=10) and control group (n=10) that received nothing. Animals were kept in standard condition. In 30 day after inducing Toxoplasma infection, 5cc blood was collected for serum protein and TAC levels. Kidney tissues of Rat in whole groups were removed and prepared for apopetosis analysis.
Results: Serum protein and kidneys weights were significantly decreased in groups that were infected with *T. gondii*, in comparison to control and onions groups. Kidneys Apopetosis in toxoplasma group significantly increased in comparison to control group (P<0.05).level of TAC was significantly increased in groups that received onion juice (P<0.05).
Conclusion: This study *showed* that *T. gondii* have significantly effect on serum protein and TAC, apopetosis and fresh onion juice returned and treated this harmful effect, so it is suggested that eating of onion is useful in toxoplasma infection.

Keywords: *Apoptosis, Allium cepa, Kidney, Protein, Toxoplasma gondii.*

Introduction

Renal failure or kidney failure describes condition in which the kidneys fail to adequately filter toxins and waste products from the blood. There are two forms of this disease: acute and chronic.Renal failure is described as a decrease in glomerular filtration rate. Biochemically, renal failure is typically detected by an elevated serum creatinine level and proteinuria (protein loss in the urine) may occur. Toxoplasmosis is a parasitic disease caused by the protozoan *Toxoplasma gondii* [1]. The parasite infects most genera of warm-blooded animals, including humans, but the definitive host is the felid (cat) family. Animals are infected by eating of 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 [2]. Up to one third of the world's human population is estimated to carry a Toxoplasma infection [3].

The Centers for Disease Control and Prevention notes that overall seroprevalence of toxoplasma infection 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% [4]. Many parasites such as *Cryptosporidium* , *Toxoplasma* , *Leishmania*, *Trypanosoma*, *Strongyloides*, *Malaria*, and schistosoma cause to severe infection in immunocompromised patients.These infections may be either acquired de novo (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 vitamin A, B, C, and E in diet can protect mammalian cells DNA from free radicals [5]. Evidences suggest that Allium cepa has antioxidative effects in rats and can

[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 apopetosis rate in renal celss, in rats infected by *Toxoplasma gondii*.

Material and Method

Plant material

Preparation of onion juice:

The underground yellowish-white bulbs of Allium 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 [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 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 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 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 rats. The rats were intraperitoneally injected with 10^7 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. However, the experimental infected rats (n =20) split to two toxoplasma infected groups ,one of this group was toxoplasma test (n=10) and the other was toxoplasma group(n=10) which received 1 cc of fresh onion juice daily , the fourth group (n=10) received just 1 cc of fresh onion juice daily [6]. 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 kidneys in control and experimental groups were immediately removed. The weights of kidneys 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.

Histopathological kidney studies:

For histological studies, kidneys were fixed in formaldehyde 10% , buffer .5µm, paraffin sections were stained with ***H&E*** and ***Masson's trichrome*** staining methods.

TUNEL analysis of apoptosis

The in-situ DNA fragmentation was visualized by TUNEL method [10]. Briefly, dewaxed kidney tissue sections were predigested with 20 mg/ml proteinase K for 20 min and incubated in phosphate buffered saline solution (PBS) containing 3 % H2O2 for 10 min to block the endogenous peroxidase activity. The sections were incubated with the TUNEL reaction mixture, fluorescein-dUTP (in situ Cell Death Detection, POD kit, Roche, Germany), for 60 min at 37°C. The slides were then rinsed three times with PBS and incubated with secondary anti-fluorescein-

POD-conjugate for 30 min. After washing three times in PBS, diaminobenzidine- H2O2 (DAB, Roche, Germany) chromogenic reaction was added on sections and counterstained with hematoxylin. As a control for method specificity, the step using the TUNEL reaction mixture was omitted in negative control serial sections, and nucleotide mixture in reaction buffer was used instead. Apoptotic germ cells were quantified by counting the number of TUNEL stained nuclei per seminiferous tubular cross section. Cross sections of 100 tubules per specimen were assessed and the mean number of TUNEL positive germ cells per tubule cross- section was calculated.

Serum analysis for biochemical studies:

Level of blood total protein was measured using kits (Merck Diagnostic Ltd, India) followed by spectrometric methods. The values were expressed in mg dL−1 in all the cases.

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

Results

Weight of individual male kidney:

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

Histopathological studies of kidneys:

Histopathological studies of kidneys showed the normal architecture including glomerulus, bowman capsule, proximal and distal tubules. Toxoplasmosis markedly disrupted the histology as evidenced by the tubular degeneration, tubular congestion, tubular dilatation ,necrosis and glomerular injuries (Figure A,B). Prevention of nephropathological injuries was determined with onion in toxoplasma treated rats (Figure C).

Results of Serum Protoien mesurment:

Effects of 1 cc fresh onion juice and Toxoplasma plus oninon juice on blood protein was shown in (Table 1). Reduces in protein level significantly increased in toxoplasma group than the control and onion groups. Administration of 1cc fresh onion juice in combination with Toxoplasma ameliorated the pathogenity of *Toxoplasma* and the level of protein was significantly reduced, as compared to that of the toxoplasma treated group. However, non significant alteration in the level of blood protein was recorded with the onion administration alone as compared to the control group (P>0.05).

Results of total antioxidant capacity (TAC) measurement in serum:

Total antioxidant capacity (TAC) was significantly higher in groups that received fresh onion juice as compared with toxoplasma group (P<0.05) (Table 1).

Table 1. The effect of the of 1cc fresh onion juice /rat on kidney weights and serum ceratinin,albomin,total protoin,urea ,TAC, MDA and kidney weight of control and toxoplasma groups in the rats.

Groups	Control	1^{cc} fresh onion juice /rat	toxoplasma	toxoplsma plus,1^{cc} fresh onion juice /rat
kidney (gr)	0.49 0.55	0.48 0.54	0.45 0.55	0.45 0.55
Serum protein	9 0.8	8 0.5	4 0.5	7 0.3
Total Antioxidant capacity, (mmol/ml)	0.80 0.55	1 0.01*	0.50 0.55*	0.60 0.55*
Percent of Apoptotic cells(%)	8 0.11	7 0.11	19 0.11	11 0.11

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

Discussion

Parasites are an important union in the rich tropical and subtropical bioecology , and depended to the climatic conditions , economic standards and lack of adequate preventive health care programmes. Toxoplasma 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 [12]. Many parasites such as cryptosporidium, toxoplasma,

leishmania, trypanosomia, strongyloides, malaria and schistosomia cause to severe infection in the immunocompromised patients.The first reports came up from South East Asia [13], where up to 18.5 % of acute renal failures (38.5% of those due to medical causes) are attributed to Plasmodium falciparum infections. Many parasitic infections lead to acute or subclinical, self-limited glomerulopathy during the early phase of immune stimulation[14]. Toxoplasmosis and schistosomiasis, malaria, filariasis, leishmaniasis, trichinosis, ecchinococcosis and trypanosomiasis cause to glomerular lesions and urinary abnormalities such as proteinuria, lymphocyturia and pyuria were accoured [14]. Acute interstitial lesions resulted by parasite-associated interstitial nephritis in many parasistis infection like kala-azar disease and heavily infiltrated with monocytes and lymphocytes, which clearly display an acute cell-mediated inflammation.Occasionally, renal function is impaired even with acute oliguric renal failure [14].

Figure A: Histopathological studies of kidneys showed the normal architecture including glomerulus, bowman capsule, proximal and distal tubules(arrow).X320,H&E.

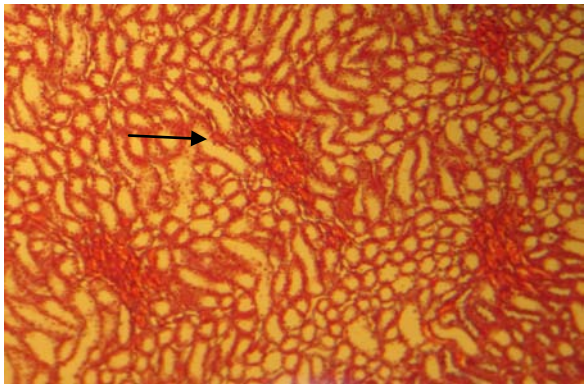

Figure B:Toxoplasmosis markedly disrupted the histology as evidenced by the tubular degeneration, tubular congestion, tubular dilatation ,necrosis and glomerular injuries (aroww),X320,H&E.

Researchers was 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 [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].

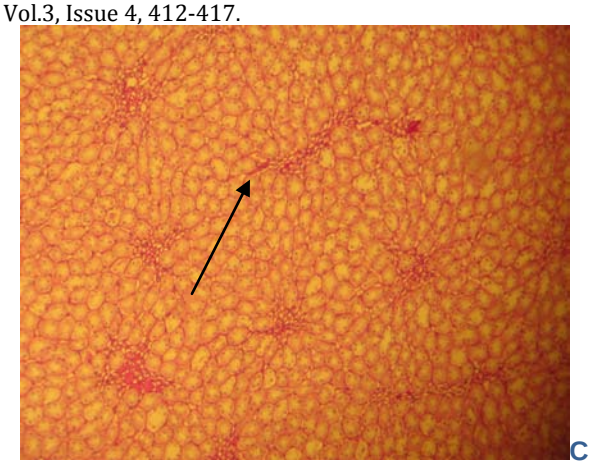

Figure C: Prevention of nephropathological injuries was determined with onion in toxoplasma treated rats (aroow).X320.H&E.

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 [21,22]. Haidari showed That Oral administration of onion at 3.5 and 7.0 mg kg(-1) day(-1) for 7 days was able to reduce serum uric acid levels in hyperuricemic rats with no significant effects on the level of this compound in the normal animals. In addition, when onion tested in vivo on rat liver homogeneities elicited significant inhibitory actions on the Xanthine Dehydrogenase (XDH) and Xanthine Oxidase (XO) activities [23]. Our results showed that administration of onion juice (1 g/rat/day) for 20 consecutive days caused a marked increase in TAC and significantly decrese in MDA, creatinine and BUN levels, as compared to respective controls and this agree with our previous research[6,24].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 [25]. Perivous study shwoed, the effect of quercetin on serum MDA was determined, but the results indicated no obvious effect of quercetin on MDA production [26,27].

In present study T. gondii was significantly reduced level of TAC showed glumerular and tubular injury and cause to hypoproteinemia, albuminemia, and reduces of reabsorbation in kidneys and in other hand our researches showed that onion fresh juice can re uptake and balance the level of TAC and have balancing rule in creatinine, BUN, protein and albumin in witch group of animals that infected with

T. gondii ,and this results in agreement with other researchers finding that showed onion can treatment hyperuricemic rats and balances the level of hepatic xanthine dehydrogenase/xanthine oxidase activities [23]. 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 results were showed that onion fresh juice could significantly increase and recovery of serum parameters such as TAC, BUN, and Protein levels in infected rats, in other study that done by McCarthy and friends study in 2003, they were showed vitamin E and selenium could resulted in trends toward increased tissue cyst number, tissue pathology, and weight loss during infection [28].

Conclusion

In our study, T. gondii have significantly effect on protein losing and cause to hypo proteinemia ,increased serum creatinin,these finding revealed fresh onion juice has strong antioxidant potensial and decreasing cell injury such as apopetosis in tubular and nephrons and can modereated harmful effect of T. gondii ,so it is suggest eating of onion is useful in infected patients.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed

CONFLICTS OF INTEREST

The authors declare that they have no competing interests