European Journal of Medicinal Plants
4(2): 135-144, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Protective Role of Onion Oil on Hepato-testicular Oxidative Damage Induced by Gamma Irradiation in Rats

O. A. Gharib[1*]

[1]Drug Radiation Research Department, National Center for Radiation Research and Technology, Atomic Energy Authority, Cairo, Egypt.

Author's contribution

The whole work was carried out by the author OAG.

Original Research Article

Received 28th September 2013
Accepted 2nd November 2013
Published 27th November 2013

ABSTRACT

Aim: Onions have potential antioxidant properties that lower the oxidative status. The Present study was aimed to investigate the antioxidant activity of onion oil in irradiated male albino rats.
Study Design: Randomized controlled experiment.
Place and Duration of Study: Experimental Animal Unit, Drug Radiation Research Department, National Center for Radiation Research and Technology, Cairo Egypt.
Methodology: Animals were divided to the following groups each of eight rats. *Control group, Onion oil group*: Rats received 200 mg/kg b.wt onion oil orally for seven days. *IRR group:* Rats were exposed to γ- ray as a fractionated dose of 9 Gy for 7 days, *Onion oil + IRR group*: Rats were administered with onion oil orally along with γ- ray exposure. At the end of this study the animals were sacrificed and the effects of onion oil against hepato-testicular oxidative damages were monitored by assaying the levels of serum alanine transferase (ALT), aspartate aminotransferase (AST), alkaline phoshatase (ALP), as well as testosterone and DHEA level. In addition both liver and testis lipid peroxidation (MDA), superoxide dismutase (SOD), and catalase (CAT) were examined.
Results: The results showed a significant increase in serum ACP, AST, and ALT activities (107.7%, 91.1% and 100.2% compared to control) with a decrease in testosterone (12% compared to control) and non- significant change in DHEA. The levels of SOD (59.31±10.67 & 0.21± 0.022), CAT (0.2081± 0.022 & 0.247±0.0453) and GSH (0.32±0.046 & 3.46± 0.476) were significantly decreased in irradiated group,

**Corresponding author: Email: drolagharib@yahoo.com*

accompanied by significant increase in both liver and testis MDA (171.2±13.69 202.2±49.34) respectively. The levels of reversal effects of irradiation were shown by *Onion oil + IRR group* in both liver and testis.
Conclusion: Data concluded that onion oil showed the reversal effects of ionizing radiation induced hepato- testicular oxidative stress.

Keywords: Onion oil; Gamma Irradiation; DHEA; Testosterone; Antioxidant Activity.

1. INTRODUCTION

Ionizing radiation is known to produce reactive oxygen species (ROS) in irradiated tissues, which are known to play roles in physiological and pathological states and are regularly produced in living organism [1]. In liver tissues highly reactive superoxide radicals and hydrogen peroxide caused due to radiation exposure could be toxic to cells by direct hit at the molecular level or indirectly by generating secondary reactive oxygen species such as hydroxyl radicals. These radicals may cause oxidative damage practically to any bio-molecule [2]. Lipids, especially polyunsaturated fatty acids (PUFAS), are the better target for such oxidative damage [3], when PUFAs react with ROS in living cells it gives MDA, which have highly cyto-toxicity and inhibitory action on protective enzymes and so act as a tumor promoter and co- carcinogenic agent [4]. Sperm produce reactive oxygen species (ROS) mostly come from their normal metabolic activity. ROS can injure mitochondria, which present in its tail in large numbers and are responsible for production of energy in the cell. Excessive ROS production in the semen however leads to deterioration of spermatozoa quality and function. Moreover, abnormal sperm and white cells may cause ROS in seminal fluid. Interestingly, ROS also manage epididymal maturation and physiological maturation of sperm, attachment to the egg and acrosome reaction [5].

Owing to the harmful effects of ionizing radiation, radiobiologists have long been paying attention in identifying novel, nontoxic, effective and convenient compound to protect humans against radiation-induced normal tissue injuries. Onion (*Allium cepa Linn*) is a major source of dietary flavonoids and have used since ancient times. Onion is one of the best agents of preventive medicine and the least expensive of all medication. It contains a power-full active antiseptic element; it is recommended in case of rheumatism, hydropsy and disorders of the liver, kidney and heart, for intestinal yeast infections, diabetes, bronchial catarrh and tuberculosis [6]. It was found that aqueous extracts of onion could offer a measure of protection against testicular oxidative injure and spermiotoxicity by possibly reducing lipid peroxidation and increasing the antioxidant defense mechanism in rats. The decrease in malondialdehyde concentrations that the researchers found indicates how the onion juice enhances testosterone production: antioxidants in onion juice neutralize free radicals in the testes [7]. Hence in the present study the prophylactic effect of onion oil against radiation exposure was investigated, using male albino rats as an experimental model.

2. MATERIALS AND METHODS

2.1 Chemicals

The onion oil and all other chemicals were obtained from Sigma Chemical (USA). Kits used in this experiments were purchased from Bio-Diagnostics (UK).

2.2 Radiation Process

Fractionated dose whole body irradiation (9 Gy as 3Gy × 3times for a week) was per-formed with rats at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt using the gamma Cell-40 biological irradiator furnished with a Caesium^{137} source (Atomic Energy of Canada LTD) with the dose rate of 0.912 rad/sec. The radiation process of animals has been carried out at the central position of the sample chamber using a special designed polyethylene plates with a polyethylene cover. This place is actually calibrated using alanine dosimeter relative to a primary standard.

2.3 Animals and Treatment

Male albino rats, each weighing 150-180 gm, were purchased from the animal breeding unit of the National research centre, Giza, Egypt. Rats were housed under appropriate conditions of controlled humidity, temperature and light. The animals were allowed free access to water and fed a standard pellet rat diet. The rats were acclimatized in the animal facility of the NCRRT -Atomic Energy Authority, Cairo, Egypt for at least one week before subjecting them to experimentation. Rats were segregated into groups of eight animals each. Each group was subjected to one of the following treatments:

2.3.1 Control animals group

2.3.1.1 Onion oil treated group

onion oil was supplied orally to a certain group of animals as a cumulative doses of 200 mg/oil kg b.wt. for seven days.

2.3.1.2 IRR group

Animals in IRR group were exposed to gamma rays as a fractionated dose of 9 Gy (3Gy × 3times) for a week.

2.3.1.3 Oil and IRR group

Animals in this group were administered orally with onion oil (200 mg/Kg bawd) along with γ-ray exposure by the fractionated dose of 9 Gy (3Gy × 3 times) for 7 days.

After 48 hours of last injection all groups were sacrificed; peripheral blood was collected, serum was obtained by centrifugation at 3000 rpm for 15 minutes and kept at -20°C until use. Liver and testis were removed and rinsed with physiological saline. A half gram of tissues were weighed and mechanically homogenized by using electrical homogenizer (Potter-Elvehjem) in a 10-fold volume of ice-cold physiological saline. The homogenate was divided into aliquoted and kept at - 70°C.

2.4 Biochemical Analysis

The blood samples were collected directly from the animals by heart puncturing. The blood samples were centrifuged using universal 16R/ Germany centrifuge at 3000 rpm for 15 min; clear serum was collected and stored in a refrigerator. The activities of ALT, AST, ACP and ALP as well as serum testosterone and DHEA level were analyzed. Liver and testis were

excised from the rat homogenization was carried out using a homogenizer (universal laboratory AID type MPW- 309, Poland). The homogenate was used to analyze MDA and GSH levels as well as CAT, SOD and GPx activities. The quantitative determination of ALP and ACP activities was done using kit according the method of Kind and King [8]. ALT and AST activities were estimated by kits according to the method of Reitman and Frankel [9]. MDA was assayed by the method of Yoshioka, *et al*. [10]. GSH content was determined using the method of Beutler *et al*. [11]. SOD activity was estimated by the method of Marklund and Marklund [12]. The activity of GPx was measured according to the method of Gross *et al.* [13]. Catalase activity was measured according to the method of Sinha [14]. Protein content was estimated according to the method of Lowery *et al.* [15]. All biochemical assays were performed with a Heλios UV/ VIS spectrophotometer (Thermo Spectronic, UK). The quantitative determination of testosterone and DHEA were done using a commercially available enzyme immunoassay (ELISA) Kits.

2.5 Statistical Analysis

To assess the significant level of influence caused by onion oil in irradiated rats, one way analysis of variances (ANOVA) followed by Tukey's multiple comparison test was used. Data are representative of 8 independent experiments carried out in triplicate. Statistical analysis was performed by using Graph-Pad software, San Diago, CA, USA) Differences were considered statistically significant when the P value was less than 0.05.

3. RESULTS

3.1 Serum Enzyme Activities

Figs. 1 &2 showed the effect of onion oil on serum ALP, ACP (Fig. 1), AST and ALT (Fig. 2) activities before radiation exposure. Serum ALP activity showed a slight significant increase recording 4.9% comparing to normal control value, while the activities of ACP, AST, and ALT increased significantly following radiation exposure. This increase was recorded as 107.7%, 91.1% and 100.2% as compared to control level respectively. Administration of onion oil before radiation was beneficial in recovering both serum ALP and ACP activity (Fig. 1). While AST (22.5%) and ALT (34%) activities were still significantly increased as compared to the normal control levels (Fig. 2).

3.2 Serum Hormonal Activity

Fig. 3 indicated the effect of radiation exposure on serum total testosterone and DHEA levels. Radiation exposure imposes a slight significant decrease on serum total testosterone that recorded -12.6% accompanied with non- significant increase as in case of DHEA level comparing with the normal control level. Administration of onion oil before radiation exposure causes a full restoration in serum testosterone level.

3.3 Hepatic Oxidative Stress and Lipid Per oxidation

Table 1 showed the toxic effects on some hepatic oxidative stress variables upon radiation exposure and subsequent treatment. A significant decrease in GSH content (- 26.13%) was shown accompanied by significant increase in MDA level (95.19%) after radiation exposure. Supplementation of onion oil results in significant recovery in GSH content and the percentage change was recorded -6.1%, while reached 44.1% as in case of MDA level and still significantly difference than the control level.

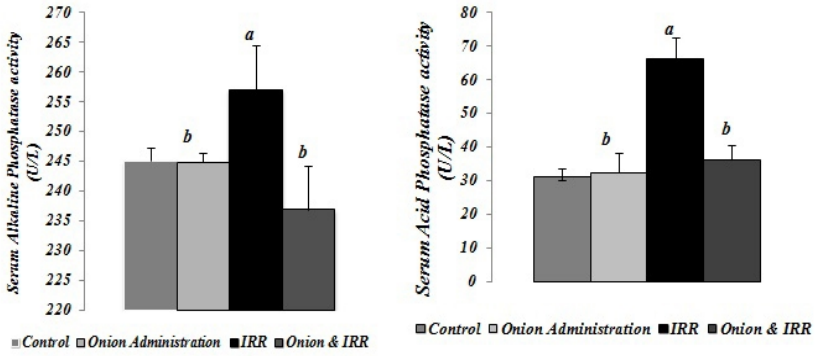

Fig. 1. Effects of onion administration and radiation exposure on serum Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP) activities (U/ L) 48 hours post last treatment (*n=8*)

Data are presented as mean (± SD). [a] Significantly different from the control group (P<0.05). [b] Significantly different from the IRR group (P<0.05). IRR: Radiation exposure with the dose of 9 Gy (3 times for one week).

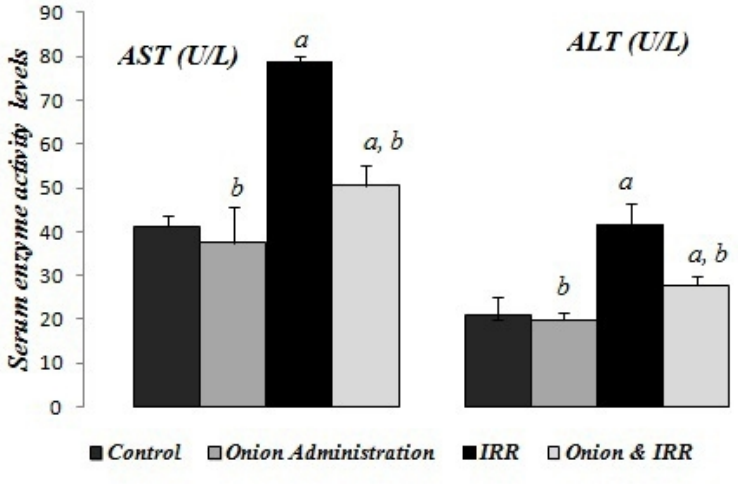

Fig. 2. Effects of onion administration and/ or radiation exposure on serum AST and ALT activities (U/ L) 48 hours post last treatment (*n=8*)

Data are presented as mean (± SD). [a] Significantly different from the control group (P<0.05). [b] Significantly different from the IRR group (P<0.05). IRR: Radiation exposure with the dose of 9 Gy (3 times for one week).

Radiation exposure imposes deleterious effects on the antioxidant enzymes in liver as evidenced by a significant decrease in GPx, CAT and SOD activities suggesting impaired antioxidant defense system. This decrease was recorded 28.89%, 27.08% and 30.5% as compared to control level respectively. The inhibited liver GPx and SOD activity showed significant protection following treatment with onion oil, while CAT activity still significantly decrease than control level (Table 1)

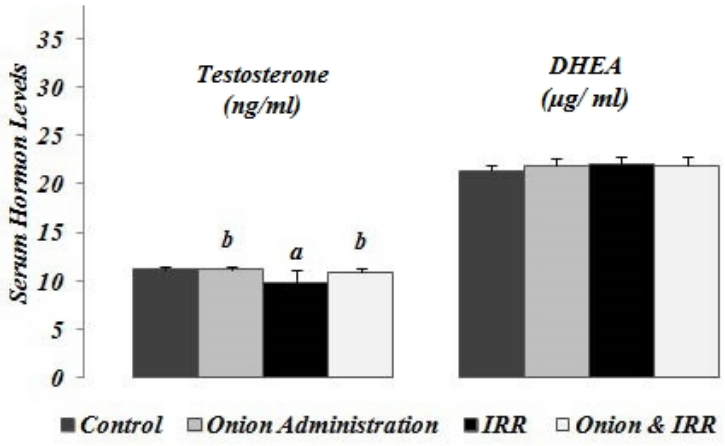

Fig. 3. Effects of onion administration and/ or radiation exposure on serum Total testosterone level (ng/ ml) and DHEA level (µg/ ml) 48 hours post last treatment (*n=8*)

Data are presented as mean (± SD). [a] Significantly different from the control group (P<0.05). [b] Significantly different from the IRR group (P<0.05). IRR: Radiation exposure with the dose of 9 Gy (3 times for one week).

3.4 Testis Oxidative Stress and Lipid Peroxidation

Exposing rats to radiation dose of 9 Gy (3 Gy X 3 times for one week) was associated with deleterious effect on the oxidative stress marker in testis as evidenced by a significant increase in MDA level by 111.75% that ameliorated by supplementation of onion oil before radiation exposure and reaching 13.73% comparing to control level.

Table 1. Effects of onion administration and/ or radiation exposure on liver tissue 48 hours post last treatment (*n=8*)

Treatment $X^- \pm SD$	Control Group	Onionoil Group	IRR Group	Onion & IRR Group
GSH (mg/gprotein)	0.4313±0.04853	0.4375±0.07906[b]	0.3186±0.04611[a]	0.4050±0.03703[b]
MDA (µmol/g protein)	87.71±12.75	85.17±12.15[b]	171.2±13.69[a]	126.4±17.83[a]
GPx (mg/min/g protein)	37.70±2.866	36.33±3.462[b]	26.81±4.162[a]	37.52±2.106[b]
SOD (U/g protein)	85.34±8.746	86.25±14.82[b]	59.31±10.67[a]	78.32±8.035[b]
Catalase ($\mu molH_2O_2$/min./ g protein)	0.288±0.0244	0.281±0.0203[b]	0.2081± 0.022[a]	0.235 ±0.0120[a]

Data are presented as mean (± SD). [a] Significantly different from the control group (P<0.05). [b]Significantly different from the IRR group (P<0.05). IRR: Radiation exposure with the dose of 9 Gy (3 Gy X3 times for one week).

Table 2. Effects of onion administration and/ or radiation exposure on testis tissues 48 hours post last treatment (*n=8*)

Treatment $X^- \pm SD$	Control Group	Onion oil Group	IRR Group	Onion & IRR Group
GSH (mg/gprotein)	5.124± 0.316	4.906± 0.517 [b]	3.457± 0.476 [a]	4.573± 0.4372 [b]
MDA (µmol/g protein)	95.49±20.29	100.4±24.57	202.2±49.34 [a]	108.6±26.97
GPx (mg/min/g protein)	10.13±0.9471	10.63±0.9789 [b]	6.066±0.5987 [a]	9.574±0.9207 [b]
SOD (U/g protein)	0.1919± 0.005	0.2081± 0.022 [b]	0.1426± 0.0162 [a]	0.1772± 0.0117 [b]
Catalase (µmolH_2O_2/min./g protein)	0.300±0.0251	0.321±0.0522 [b]	0.247±0.0453[a]	0.311±0.0203[b]

Data are presented as mean (± SD). a Significantly different from the control group (P<0.05). b Significantly different from the IRR group (P<0.05). IRR: Radiation exposure with the dose of 9 Gy (3 Gy X 3 times for one week).

Radiation intoxication imposes deleterious effect on antioxidant enzymes in testis as evidenced by a significant depletion in GSH content as well as GPx, SOD and catalase activities. The depletion of these parameters was 32.53%, 40.118%, 25.69% and 17.67% respectively as compared to control level. Administration of onion oil showed a significant protection against radiation damage.

4. DISCUSSION

Onions *(Allium Cepa L.)* contain chemical compounds such as phenolic, flavonoids as well as quercetin and its glycosides quercetin which are the most abundant flavonoids in onions [16]. Onions are rich in vitamin C, vitamin B and folic acid that have prospective anti-inflammatory, anti- cholesterol, anti- cancer and antioxidant properties [17]**.** They also contain allyl propyl disulphide and diallyl disulphide and other compounds that lower the oxidative stress status [18]. Foods' antioxidants and vitamins consumed by animals, such as quercetin, vitamin C, vitamin B and vitamin E, could recover sperm health parameters and testicular androgenesis. The results of Khaki *et al.* 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 Toxoplasmosis infected rats [17].

The majority of the toxic effects of ionizing radiation to liver and testis are due to the generation of ROS. This was indicated by the elevation of ALT, AST, ALP and ACP activities with a decrease in testosterone level in the present data. To overcome such events, living cells are equipped with full and integrated endogenous non- enzymatic and enzymatic antioxidant systems such as GSH as well as SOD, CAT and GPx respectively [19].

Free radical generated by irradiation also react with unsaturated lipid generating hydroperoxides, which in turn can induce changes in the lipid bilayer thereby altering the membrane permeability and inducing lipid peroxidation [20] that may lead to a decrease in testosterone level [21] as shown in the present study. Onion oil reduced the radiation-induced lipid peroxidation in both testis and liver and cause a full restoration in testosterone level since onion oil found to be contained vitamin C, B and folic acid that have potential

antioxidant properties [17]. Flavonoids also may exert their antioxidant activity via scavenging some radical species by acting as chain-breaking antioxidants [22]. The depletion in GSH after exposure to γ- radiation may be due to the reaction of GSH with free radicals that associate to produce GSSG [1]. Furthermore, normal synthesis / repair of GSH will be impaired due to damage to DNA and membrane [23].

Oxidative stress arising as a result of over- production of radical and non- radical ROS is inactivated by SOD and CAT [24]. Treatment with onion oil in the present study effectively reduced the increase of lipid peroxidation and enhanced the level of antioxidant enzymes CAT, SOD, GPx in both testis and liver [25]. These results indicate protective effects of onion oil against oxidative stress. By increasing the GPx and SOD activities, which remove peroxides and superoxides; quercetin, which is the most abundant flavonoids in onions might prevent the accumulation of ROS by trapping them [22]. These modulations in the antioxidant enzyme system might be associated with reduced risk of testis and liver cancer. Lipid peroxides cause damage to cellular macromolecules by generation of ROS [26], which is considered to enhance carcenogenesis [27]. Sparnin *et al.* suggested that the protective effect of diallyl sulphid might be caused by increased activity of GST, which catalyze conjunction of electrophilic compounds of the cell following treatment with diallyl sulphid [28]. This incident is valuable to the detoxification and antioxidation capabilities of hepatocytes [29], as they protect cells from the toxic effects of ROS.

5. CONCLUSION

The present data demonstrate that onion oil inhibit the oxidative injury in testis and liver and imply that antioxidant enzymes can be used on prevention of radiation damages.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Author has declared that no competing interests exist.

REFERENCES

1. Prabhakar KR, Veerapur VP, Bansal P, Parihar VK, Reddy Kandadi M, Bhagath Kumar P, Priyadarsini KI, Unnikrishnan MK. Antioxidant and radioprotective effect of the active fraction of Pilea microphylla (L.) ethanolic extract. ChemicoBiological Interactions. 2007;165:22–32.

2. Ogony J, Matthews R, Anni H, Shannon K, Ercal N. The mechanism of elevated toxicity in HepG2 cells due to combined exposure to ethanol and ionizing radiation. J Appl Toxicol. 2008;28(3):345-55.
3. Douillet Cy, Chruz C, Maroncles C, Kergonou JF, Renaud S, Ciacatti M. High dosage vitamin E effect on oxidative status and serum lipid distribuation in Streptozotocin induced diabetic rats. Biochem. Medi. Metab. Biol.1993;50:265.
4. Taysi S, Polat F, Gul M, Sari R, Bakan E. Lipid peroxidation, some extracellular antioxidant enzymes in serum of patients with rheumatoid arthritis. Rheumatol. Int. 2002;21:200.
5. Koppers AJ, De Iuliis GN, Finnie JM, McLaughlin EA, Aitken RJ. Significance of mitochondrial reactive oxygen species in the generation of oxidative stress in spermatozoa. J Clin Endocrinol Metab. 2008;93(8):3199-207.
6. Yang J, Meyers KJ, Van der Heide J, Lui RH. J. Agric food Chem. 2004;52(22): 6787-6793.
7. Mudathir OKF, Suru SM, Fafunso MA, Obioha UE, Faremi TY. Protective roles of onion and garlic extracts on cadmium-induced changes in sperm characteristics and testicular oxidative damage in rats. Food Chem Toxicol. 2008;46(12):3604-11.
8. Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolyzed phenol with aminopyrines. J Clin Path.1954;7:322.
9. Retiman S, Frankel SA colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957;28:56- 63.
10. Yoshioka T, Kawada K, Shimada T, Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Am J Obstet Gynecol 1979;135(3):372-76.
11. Beutler E, Duran O, Kelly BM: Improved method of blood glutathione. J Lab Clin Med 1963;61(5):852-855.
12. Marklund S, Marklund G. Involvement of the superoxide anion radical in the auto-oxidation of pyrogallol and convenient assay for superoxide dismutase. Eur.J.Biochem. 1974;47:69-474.
13. Gross RI, Bracci R, Rudolph N, Schroeder E, Kochen J. Hydrogen peroxide toxicity and detoxification in the erythrocytes of new born infants. Blood. 1967;29(4):481-493.
14. Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972;47:389-94.
15. Lowery OH, Rosebrough AJ, Farr AL, Randall RJ. Protein measurements with folin phenol reagent. J.Biol. Chem, 1951;193:265-269.
16. Hollman PCH, de Vries JHM, van Leeuwen SD, Mengelers JB, Katan M.B. Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. Am. J. Clin Nutr. 1995;62:1276—1282.
17. Khaki A, Farzadi L, Ahmadi S, Ghadamkheir E, Khaki A, shojaee S, Sahizadeh R. Recovery of spermatogenesis by Allium cepa in Toxoplasma gondii infected rats African Journal of Pharmacy and Pharmacology. 2011;5(7): 903-907 Available online http://www.academicjournals.org/ajpp.
18. Jeon GI, Shin MJ, Lee KH, Park E. Effect of onion juice supplementation on antioxidant status in participants with mild hypercholesterolemia. Food Sci. Biotechnol. 2013;22(s):227- 231.
19. Karbownik RJ, Reiter. Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. Proc. Soc. Exp. Biol. Med. 2000;225:9–22.
20. Parasassi T, Giusti AM, Gratton E, Monaco E, Raimondi M, Ravagnan G, Sapora O. Evidence for an increase in water concentration in bilayers after oxidative damage of phospholipids induced by ionizing radiation. Int J Radiat Biol. 1994;65(3):329–334.
21. Gharib OA, Ibrahim NK. Oxidative damage in testes induced by 950 MHz stimulating cellular phone. Isotope and Rad. Res. 2010;42(4):941- 953.

22. McAnlis GT, McEneny J, Pearce J, Young IS. Absorption and antioxidant effects of quercetin from onions, in man. Eur J Clin Nutr. 1999;53:92–96.
23. Navarro-Antolin J, Hernandez-Perera O, Lopez-Ongil S, Rodriguez-Puyol M, Rodriguez- Puyol D, Lamas S. CsA and FK506 up-regulate eNOS expression: role of reactive oxygen species and AP-1. Kidney Int Suppl. 1998;68:S20–S24.
24. Vang O, Rasmussen BF, Andersen O. Combined effects of complex mixtures of potentially anti-carcinogenic compounds on antioxidant enzymes and carcinogen metabolizing enzymes in the rat. Cancer Lett. 1997;114:283-6.
25. Khaki P, Bidehendi MS, Vand e Yousefi J. Prevalence of Leptospirosis in Iran. 4th Scientific Meeting of the Intrnational Leptospirosis Society. November 14-16. Chiang Mai, Thailand. 2005;179.
26. Chung FL, Wang M, Rivenson A, Iatropoulos MJ, Reinhardt JC, Pittman B, et al. Inhibition of lung carcinogensis by black tea in Fischer rats treated with a tobacco-specific carcinogen: caffeine as an important constituent. Cancer Res. 1998;58:4096-101.
27. Prasad S, Kalra N, Shukla Y. Modulatory effects of diallyl sulfide against testosterone-induced oxidative stress in Swiss albino mice Asian J Androl. 2006;8(6):719-723.
28. Sparnins VL, Barany G, Wattenberg LW. Effects of organosulfur compounds from garlic and onions on benzo(a) pyrene-induced neoplasia and glutathione S-transferase activity in the mouse. Carcinogenesis. 1988;9:131-4.
29. Thomson M, Ali M. Garlic (*Allium sativum*): a review of its potential use as an anti-cancer agent. Curr Cancer Drug Targets. 2003;3:67-81.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=362&id=13&aid=2616