



Assessment of the effects of red onion (*Allium cepa* Linn) juice on semen oxidative status compared to Zn sulfate in rats

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Abstract

The aim of the present study was to investigate effects of onion juice on semen values of malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx) and total antioxidant status (TAS) compared to Zn sulfate in rats. One hundred and sixty-two Wistar male rats were randomly allocated into 9 treatment groups (each including 3 groups and 6 replicates). Group 1 served as control and received distilled water. In group 2, animals received 1 cc fresh onion juice. In group 3, rats were offered 2 cc fresh onion juice. Group 4 drenched 15 mg/kg zinc (Zn) sulfate. In group 5, rats were treated with 30 mg/kg Zn sulfate. Group 6 was offered 1cc fresh onion juice + 15 mg/kg Zn sulfate to experimental animals. In group 7, 1 cc fresh onion juice + 30 mg/kg Zn sulfate was provided to rats. Group 8 consumed 2 cc fresh onion juice + 15 mg/kg Zn sulfate. In group 9, animals provided 2 cc fresh onion juice + 30 mg/kg Zn sulfate. All groups were given treatments orally and *ad libitum* access to chow pellets and fresh water. After 4 weeks semen samples in cauda epididymis were used to determine MDA, SOD, GPx and TAS levels. According to the data, sole onion juice significantly decreased semen MDA level ($P < 0.05$). Also, a combination between the administration of onion and Zn significantly attenuated sperm MDA ($P < 0.05$). Sperm GPx level in the co-administration of onion and Zn was significantly altered ($P < 0.05$). Results suggest that presumably onion juice protects from semen oxidation in rat testes.

Keywords: onion juice, oxidative enzymes, rat, semen, Zn sulfate.

Introduction

One of the main health problems in couples is infertility and approximately 30% of the problems are related to the males (Khaki *et al.*, 2009; Lee *et al.*, 2012; Barkhordari *et al.*, 2013). Recently, a wide number of plant-derived pharmaceutical products are being used in traditional medicine because of their beneficial

properties (Yama *et al.*, 2011; Ghiasi Ghalehkandi *et al.*, 2013). Plants are a rich source of a wide variety of secondary metabolites such as flavonoids, tannins, terpenoids, alkaloids among others (Hassanpour *et al.*, 2011). Nowadays, worldwide interest on folk medicine has increased. People desire to consume much more medicinal plants due to their therapeutical properties (Kishk and Elsheshetawy, 2013; Yousif *et al.*, 2013). The edible *Allium* species, garlic (*A. sativum* L.) and onion (*A. cepa* L.) have long been used as food ingredients and medicine (Atmaca, 2003; Durrani *et al.*, 2010; Kim *et al.*, 2011). Numerous health benefits have been identified which attract researchers to investigate the validity of the medical properties of these plants. Their bulbs and corms are magnificently nutritious and have therapeutic effects such as anti-diabetic, anti-atherosclerotic, anti-thrombotic, anti-hypertensive, anti-hyperlipidemic, anti-inflammatory, antioxidant and even anti-neoplastic effects (Bozcuk *et al.*, 2011; Khaki *et al.*, 2012). Anti-oxidative properties of aqueous onion juice have been proved in studies conducted on animals (Khaki *et al.*, 2009, 2012). It is claimed that the phytochemical composition of onions changes depending on the specific variety and cultivation methods but it is suggested that the phytochemical ratio is higher in the red onion than other species (Lee *et al.*, 2012). Antioxidants are a wide group of bioactive compounds which resist cell oxidation (Erguder *et al.*, 2007). It was reported that fresh onion juice has a positive effect on sperm health and spermatogenesis in rat (Khaki *et al.*, 2012).

Zinc is the second most abundant transition metal, after iron, in the body. Zinc is an essential trace element and plays a key role in the immune system, growth, protein, DNA synthesis and reproduction (Egwurugwu *et al.*, 2013). In the male reproductive system, zinc is essential for several functions including: capacitation, acrosome reaction and protection of the testis against degenerative changes (Wong *et al.*, 2002). It was reported that Zn supplementation promotes decrease in abnormalities during spermatogenesis which increase sperm viability (Babaei and Abshenas, 2013; Kheradmand *et al.*, 2013).

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Reactive oxygen species (ROS) have an essential effect in human reproduction. Several antioxidants related to the ROS detoxification system include superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GPx; Chi *et al.*, 2008). Polyunsaturated fatty acids (PUAFAs) are highly concentrated in spermatozoa which are vulnerable to be attacked by ROS. Malondialdehyde is the end product of lipid peroxidation, where elevation in testicle MDA levels is a sign of lipid peroxidation and leads to infertility (Hsieh *et al.*, 2006). Glutathione peroxidase is an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage and lipid peroxidation (Lee *et al.*, 2012). GPx impresses its role by protecting sperm against peroxidative damage (Hsieh *et al.*, 2006). It is suggested that there is a correlation between excessive ROS generation in semen and infertility (Hsieh *et al.*, 2006). Quercetin is the chief flavonoid compound in onions. It is suggested that it protects DNA and other important molecules from oxidation and probably protects sperm against oxidation and improves fertility in men (Khaki *et al.*, 2009; Lee *et al.*, 2012). Furthermore, researchers have shown that onion juice improves sexual behavior in rats (Khaki *et al.*, 2012; Paccola *et al.*, 2013). However, based on scant evidence, the involvement of onion juice on sperm health remains controversial. In this regard, based on previous work on antioxidant properties of onion, our hypothesis was to investigate the possible role of onion juice on fertility. Thus, the present study was conducted to evaluate and compare the effects of fresh onion juice and Zn supplementation on semen MDA, SOD, GPx and total antioxidant status (TAS) in rats. We used Zinc sulfate to examine the effects of Zn on semen. Then we examined the possible effects of co-administration of fresh onion juice and Zn sulfate on rat semen.

Materials and Methods

Animals

To survey possible effects of fresh onion (*A. Cepa. Linn*) juice on semen values of MDA, SOD, GPx and TAS activities compared with Zn sulfate supplementation, a hundred and sixty-two male Wistar albino rats (230-250 g) were purchased from Razi Vaccine and Serum Research Institute, Iran, and randomly allocated into 9 treatment groups (each including 3 groups and 6 replicates). The rats were housed individually in stainless steel wire-bottomed cages and resided under standard laboratory conditions according to European community suggestions for laboratory animals at a temperature of 23.1-25.8°C, relative humidity of 55-60% and a 12 h light period (starting at 8:00 AM). All animals had *ad libitum* access to chow pellets (Azarbayjan Co. Iran) and fresh water.

Plant material

The *A. Cepa. Linn* (red onion) was obtained from Ilkhchi-Tabriz, East Azarbayjan province, Iran, in August 2012. The samples of *A. Cepa. Linn* were characterized at the Division of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Iran. Onion juice was obtained through a fruit juice extracting machine before the experiments and prepared in pyrogen-free bottles (Khaki *et al.*, 2009, 2012).

Analysis of onion juice

The flavonoid components of onion juice were determined through the Shinoda test (Yousef, 2005) at the Tehran University of Medical Sciences. Quercetin as chief flavonoid in onion was determined using qualitative thin-layer chromatography (TLC). To this end, 10 ml of fresh onion juice was dried in a vacuum container and the resulting residue dissolved in 1 ml of methanol. Methanolic solution (20 ml) was placed on a silica gel plate (10 x 20 cm, silica gel 60 GF254, Merck, Darmstadt, Germany) by EtOAc/MeOH (80:20) solvent system. The vehicle was quercetin, Sigma chemical Co. (St. Louis, MO, USA). Then after developing and drying, 2% AlCl₃ solution in methanol was used to spray the TLC plate. Yellow spot was the recognition factor of quercetin in the onion samples at RF = 0.6. Quercetin was separated via preparative TLC on silica gel and quantitative determination of quercetin carried out with the LIAISON analyzer (DiaSorin LIAISON® XL, Italy). Quercetin levels were determined using pure quercetin standard curve in 370 nm. According to the result, the quercetin content of experimental fresh onion was 11.02 mg per 100 g.

Experimental procedure

Onion juice (1 or 2 cc) was drenched to rats on a daily basis. Zinc sulfate was purchased from Merck (©Merck KGaA, Darmstadt, Germany) and different dosages of Zn sulfate (15 and 30 mg/kg) were dissolved in water and orally administrated to animals. Doses were calculated based on our previous pilot studies (Khaki *et al.*, 2009, 2012; Ghiasi Ghalehkandi, 2012; Ghiasi Ghalehkandi *et al.*, 2012a, b). Control animals were given distilled water.

On the first week of the study, all groups received a basal diet in order to adapt to experimental conditions, then groups were divided as follows:

- Group 1: basal diet + distilled water (control)
- Group 2: basal diet + 1cc fresh onion juice,
- Group 3: basal diet + 2cc fresh onion juice,
- Group 4: basal diet + 15 mg/kg zinc sulfate complement,
- Group 5: basal diet + 30 mg/kg zinc sulfate complement,



Group 6: basal diet + 1cc fresh onion juice + 15 mg/kg zinc sulfate complement,

Group 7: basal diet + 1cc fresh onion juice + 30 mg/kg zinc sulfate complement,

Group 8: basal diet + 2cc fresh onion juice + 15 mg/kg zinc sulfate complement,

Group 9: basal diet + 2cc fresh onion juice + 30 mg/kg zinc sulfate complement.

Experimental animals were treated during 4 weeks.

Surgical procedure

At the end of the study, rats fasted overnight and were intraperitoneally (i.p) injected with pentobarbital (40 mg/kg). Peritoneum on each animal was opened by an incision and testes were taken out. Semen samples were collected from the Cauda epididymis and homogenized in 10% (W/V) ice-cold buffer (0.1 M phosphate buffer, pH 7.4 + 150 mM KCl). The homogenate was centrifuged at 9000 rpm for 20 min to obtain a supernatant which was used for SOD, MDA, GPx and TAS estimations (Sharma *et al.*, 2012). Then, animals were euthanized using CO₂ gas in a 2 h period. All experimental procedures were done at 10-12 AM. Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory animals by the National Institutes of Health (USA) and the current laws of the Iranian government. All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals to investigate experimental pain in animals (Zimmermann, 1983). All protocols for animal experimentation were approved by the institutional animal ethical committee in Islamic Azad University, Shabestar Branch, East Azarbayjan, Iran.

Semen biochemical assay

Malondialdehyde

Malondialdehyde is a standard to determine free radical damage. The detecting kit was purchased from Randox (Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom). Malondialdehyde was formed as an end product of lipid peroxidation and treated with thiobarbituric acid (TBA) to produce a colored product that was measured at 532 nm (Placer *et al.*, 1966).

Superoxide dismutase

SOD detecting kit was purchased from Randox (Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom). The role of SOD is to accelerate the dismutation of the toxic superoxide radical (O₂), produced during the oxidative energy processes, to

hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye detectable at 505 nm (Woolliams *et al.*, 1983).

Glutathione peroxidase

The commercial kit was obtained from Randox (Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom). According to this method, GPx catalyses the oxidation of glutathione and in presence of glutathione reductase and NADPH, oxide glutathione converts to reduced form by changes in oxidation of NADPH to NADP⁺ in absorbance at 340 nm (Paglia and Valentine, 1967).

Total antioxidant status

The total antioxidant status detecting kit was obtained from Randox (Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom; Cat. no. NX2332). Antioxidants added to samples cause suppression in color production which was measured at 600 nm (Miller *et al.*, 1993).

Statistical analysis

This study was performed as a factorial 3 x 3 experiment (3 levels of fresh onion juice and 3 levels of zinc sulfate complement). Data were expressed as mean values ± SEM by a one-way analysis of variance using the general linear models (GLM). All statistical analyses were performed using SAS (version 9.1). When significant difference among the means was found, means were separated using Duncan's multiple range tests. P < 0.05 was considered a significant difference between groups. The result of the analysis of variance according to the model is

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where,

Y_{ijk} = all dependent variable

μ = overall mean

α_i = the fixed effect of onion levels (i = 1, 2, 3)

β_j = the fixed effect of zinc sulfate levels (j = 1, 2, 3)

e_{ijk} = the effect of experimental error

Results

The results obtained in this study are summarized in Table 1. According to our results, the sole administration of onion juice (1 cc) for 4 weeks, significantly decreased semen MDA levels compared to the control group in rats (P < 0.05) but there no significant difference was observed between groups that received 1 or 2 cc of fresh onion juice (P > 0.05). Furthermore, Zn sulfate supplementation had no significant effect on semen MDA



concentrations ($P > 0.05$). Co-administration of fresh onion juice (1 cc) and Zn sulfate (15 and 30 mg /kg) significantly diminished semen MDA levels compared to control group in rats ($P < 0.05$). In addition, there was no significant effect on combined administration of fresh onion juice and Zn sulfate between groups ($P > 0.05$).

Regarding SOD levels in rat semen, there was no significant effect on semen SOD concentrations after administration of sole onion juice or Zn sulfate in experimental animals. Similar results were detected after co-administration of fresh onion juice and Zn sulfate on semen SOD in rat ($P > 0.05$).

The effects of onion juice and Zn sulfate on semen GPx levels in rat are shown in the Table 1. Pre-treatment with fresh onion juice (1 or 2 cc) had no

effect on semen GPx level in rats ($P > 0.05$). In contrast, the administration of Zn sulfate (15 or 30 mg /kg) had no significant effect on semen GPx concentrations compared to the control group ($P > 0.05$). Interestingly, co-administration of onion juice and Zn sulfate caused a significant change in semen GPx concentrations in rats ($P < 0.05$).

No significant fluctuations were detected on semen TAS levels post onion juice administration in rats ($P > 0.05$). Moreover, a single administration of Zn sulfate for 4 weeks had no effects on semen TAS levels ($P > 0.05$). Furthermore, co-administration of onion juice and Zn sulfate had no significant effect on semen TAS concentrations in rats ($P > 0.05$).

Table 1. Assessment of different levels of fresh onion (*A. Cepa. Linn*) juice on semen values of Malondialdehyde, Superoxide dismutase, Glutathione peroxidase and Total antioxidant status compared with Zn sulfate supplementation in rats.

	MDA (nmol/ml)	SOD (IU)	GPx (IU)	TAS (mmol/ml)	
Onion (cc)					
0 (control)	232.50 ^a	182.50	7241.66	18.25	
1	149.16 ^b	174.16	7175.00	13.33	
2	185.00 ^{ab}	185.83	7133.33	14.75	
P-value	0.06	0.73	0.31	0.10	
SEM	21.51	10.70	47.62	1.41	
Zn sulfate supplementation (mg/kg)					
0 (control)	197.50	178.33	7158.33	15.25	
15	162.50	176.66	7275.00	14.08	
30	206.66	187.50	7116.66	17.00	
P-value	0.35	0.75	0.10	0.40	
SEM	21.51	10.70	47.62	1.41	
Combined administration					
Onion (cc) Zn sulfate supplementation (mg /kg)					
0	0	260.00 ^a	185.00	7225.00 ^{ab}	18.25
	15	217.50 ^{ab}	155.00	7375.00 ^a	15.25
	30	220.00 ^{ab}	207.50	7125.00 ^{ab}	21.25
1	0	120.00 ^b	172.50	7200.00 ^{ab}	12.75
	15	127.50 ^b	182.50	7175.00 ^{ab}	13.25
	30	200.00 ^{ab}	167.50	7150.00 ^{ab}	14.00
2	0	212.50 ^{ab}	177.50	7050.00 ^b	14.75
	15	142.50 ^{ab}	192.50	7275.00 ^{ab}	13.75
	30	200.00 ^{ab}	187.50	7075.00 ^b	15.75
P-value	0.054	0.44	0.053	0.87	
SEM	37.26	18.54	82.49	2.55	

MDA: malondialdehyde, SOD: superoxide dismutase, GPx: glutathione peroxidase, TAS: total antioxidant status, Zn: zinc. SEM: standard error mean. Different letters (^{a,b}) indicate significant differences between treatments ($P < 0.05$). Data are presented as mean \pm SEM.

Discussion

To our knowledge this is the first study to investigate effect of fresh onion (*A. Cepa. Linn*) juice compared to Zinc sulfate supplementation on semen

oxidation in rats. This research revealed the role of fresh onion juice on semen MDA, SOD, GPx and TAS in rats. The result data indicate that pre-treatment with onion juice decreased epididymal MDA levels in rat semen. Sperm in the epididymis are vulnerable to



oxidative damage during maturation and the storage stage. It has been proven that ROS have a key effect on sperm maturation and capacitation, and high ROS production leads to sperm dysfunction (Hsieh *et al.*, 2006). Seminal plasma is endowed with frequent enzymatic antioxidants that include SOD, GPx, MDA (Fingerova *et al.*, 2007). Malondialdehyde is the end product of lipid peroxidation and an elevation in testicle MDA level is a sign of lipid peroxidation. So an increase in MDA levels has irreversible effects on sperm fertility and leads to infertility (Hsieh *et al.*, 2006).

On the other hand, major loss of sperm function is the result of lipid peroxidation. This phenomenon leads to cell death through damage on the cellular plasma membrane. Malondialdehyde is an index for the determination of lipid peroxidation and sperm function. Previously, Verma and Kanwar (1999) revealed that promoted MDA levels are an indicator for pathologic lipid peroxidation of sperm. It is reported that oral administration of fresh onion juice (3 cc/daily) for 4 weeks meaningfully decreased serum MDA levels in Wistar rats (Khaki *et al.*, 2012). The same reduction on MDA levels is demonstrated in male rats treated with ginger (Morakinyo *et al.*, 2008) and onion (*A. cepa*; Ige and Akhigbe, 2012). The result of the current study was parallel to previous reports. The possible mechanism to distinguish the role of onion on MDA is that onion is an anti-oxidant substance and perhaps prevents free radical generation in the semen (Amin and Hamza, 2006; Bóné and Janszky, 2006; Khaki *et al.*, 2009). Also, we used Zn sulfate to examine the effects of Zn on semen MDA. No curative effect was observed on semen MDA levels in Zn treated animals. Zinc is an essential element for normal male reproductive function e.g. adequate testosterone production (Glenville, 2008). Furthermore, it is revealed that Zn suppresses toxic effects of over dose copper consumption on sperm quality in rats (Babaei and Abshenas, 2013). The Zn level is greater in seminal plasma than serum which dictates the possibility of Zn as an anti-oxidant on semen. Hence, some reports revealed that elevated Zn levels promote sperm motility (Talevi *et al.*, 2013).

Then we examined the possible interaction modulatory effects of fresh onion juice and Zn sulfate on semen MDA in rats. In this study, co-administration of fresh onion juice and Zn sulfate diminished semen MDA levels in rats. Numerous researches have investigated the effect of antioxidants on semen quality but the direct action of antioxidants on sperm physiology is still controversial (Talevi *et al.*, 2013). In agreement with previous studies our results confirmed the potent antioxidant role of onion on the rat semen. We think quercetin as chief flavonoid in the onion might decrease semen MDA levels by altering ROS production in testes, but the direct mechanism is still unknown (Khaki *et al.*, 2012).

To assume the antioxidant effects of the onion

juice and Zn we studied their effect on semen SOD levels in rat. Our data suggest that fresh onion juice and Zn, as well as their combined administration had no effects on SOD. Superoxide dismutase is a cellular antioxidant defense system and its level increases in oxidative stress. Previously, Suru (2008) reported Cadmium-induced oxidative stress as an attenuation mechanism of renal SOD levels in rats. Interestingly, pre-treatment with oral onion and garlic extract (0.5 ml and 1.0 ml/100 g per day, respectively) significantly amplifies renal SOD levels as dose-dependent in rat. A similar result is detected on antioxidant effects of onion to increase SOD activities in carbon tetrachloride-induced toxicity (El Demerdash *et al.*, 2005). Based on our study, we think quercetin (main flavonoid in the onion) is responsible for altering semen SOD. Main superoxides (OH and H₂O₂) are responsible for oxidative stress damage in mice testis. In this regards, quercetin supplementation significantly restored the depletion level of SOD and GPx in 4-nitro-*m*-cresol (PNMC)-induced oxidative stress in rat testes (Bu *et al.*, 2012; Lee *et al.*, 2012).

In this study we determined effects of onion, Zn sulfate and their possible interactions on semen GPx status. Neither sole onion nor Zn sulfate had any change on semen GPx activity. One of the effective strategies to cure infertility is supplementation of diets with trace elements such as Zn. It should be noted that a physiological dosage of Zn sulfate must be taken, and higher doses are toxic (Egwurugwu *et al.*, 2013). In our previous study, we observed that gavage of Zn sulfate (15 mg per day/4 weeks) had amplified sperm viability and quantity (Ghiasi Ghalehkandi *et al.*, 2012b). Consistent with previous reports, co-administration of onion juice and Zn sulfate caused a slight but not significant increase in semen GPx concentrations in rats. Glutathione peroxidase could dispose hydroperoxide and other ROS. GPx has an undeniable role in sperm maturation where low GPx activity results in decreased fertility capacity. Also, a positive correlation exists between GPx concentration and asthenozoospermia where GPx improves sperm motility by catalyzing ROS (Dandekar *et al.*, 2002). Glutathione peroxidase is related to the balance between the GSSG (reduced form of glutathione) and GSH (oxidized form of glutathione) through the interaction with GPx. Reduced glutathione can neutralize hydroxyl radicals and detoxify the peroxides (Hsieh *et al.*, 2006). Antioxidant activity of aqueous extracts on semen GPx are quite different. Recently, Asadpour *et al.* (2013) reported that oral administration of aqueous garlic extract (400 mg/kg) was not able to improve GPx in lead-induced oxidative stress in rats. It seems, because of complex interactions between ROS and various antioxidants, that seminal GPx is not a truthful factor to investigate fertility (Hsieh *et al.*, 2006). In agreement with those studies, we think that further investigation is needed to clarify possible mechanisms by which antioxidants act on seminal GPx.



Finally, we studied the effects of onion juice on semen TAS activity in rats. Our present study revealed no meaningful change observed on semen TAS in rats treated for 4 weeks. Previously, Khaki *et al.* (2012) reported that serum TAS was significantly higher in onion (3 cc/4 weeks) treated male Wistar rats. In our previous study, we found that the administration of fresh onion juice (1 cc per day/4 weeks) significantly improved sperm viability and quantity (Ghiasi Ghalehkandi *et al.*, 2012b). In recent years, researchers measured SOD, MDA and GPx as an indicator for oxidative stress-associated with tissue damage and antioxidant defense but TAS was designed to measure whole antioxidants capacity instead of a single antioxidant (Kusano and Ferrari, 2008; Kim *et al.*, 2011). There is a positive correlation between oxidative stress, TAS and apoptosis (Bu *et al.*, 2012). It is reported that quercetin (onion main antioxidant) significantly declines epididymal sperm apoptosis and serum ROS levels in rat (Khaki *et al.*, 2009). Consistent with several lines of evidences and based on our knowledge, it seems that fresh onion juice protects sperm against oxidation in rat testes. In conclusion, we recommend further researches to clarify the direct interaction between antioxidants and semen MDA, SOD, GPx and TAS activity in reproductive physiology. Additionally, merit studies are needed to distinguish their potential for clinical use in clinical trials.

Conflicts of interest

The authors report no conflicts of interest.

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