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**ORIGINAL ARTICLE**

# Role of Onion (*Allium cepa. Linn*) Juice on Serum Constituents in Rats Exposed to Zn sulfate

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**ABSTRACT**
*The main porpoise of current research was to investigate effects of fresh onion juice on serum magnesium (Mg) and iron (Fe) compared with zinc (Zn) sulfate in rats. A hundred and sixty-two male Wister rats randomly allocated into 9 treatment groups (each include 3 groups and 6 replicate). In group 1, animals drenched water. In group 2, 1cc fresh onion juice was offered. In group 3, rats were received 2cc fresh onion juice. Group 4 offered 15 mg/kg Zn sulfate complement. In group 5, 30 mg/kg Zn sulfate complement delivered to rats. In group 6, animals nourished with 1cc fresh onion juice + 15 mg/kg Zn sulfate complement. In group 7, n1cc fresh onion juice + 30 mg/kg zinc sulfate complement provided to rats. Group 8 consumed 2cc fresh onion juice + 15 mg/kg Zn sulfate complement. In group 9, animal gavage 2cc fresh onion juice + 30 mg/kg Zn sulfate. All animals had free access to chow pellet. Animals were kept 1 week for adaptation to experimental condition, and then received treatments for next 4 weeks. At the end 4th week, after 12 hours fasting period, blood samples were taken and serum Fe and Mg levels determined. According to the data, different levels of sole fresh onion juice (1 and 2 cc) or Zn sulfate (15 and 30 mg/kg) and their co- administration had no significant effects on serum Fe and Mg levels (P>0.05).*
***Key words:*** *Onion juice, Zn sulfate, Fe, Mg, Rat*

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## INTRODUCTION

For centuries consumption of fruits and vegetables has been attributed to beneficial health effects. Ames and Gold [1] stated that approximately one-third of cancer risk in humans could be attributed to diet. Epidemiological studies have suggested that those persons in the lowest quartile of fruit and vegetable consumption have twice the risk of cancer as do those in the highest consumption quartile [2]. *Allium cepa* (onion) has a beneficial effect on disease treatment worldwide and has been used since ancient times as a medicinal and food source. The biological action of Allium products is ascribed to organosulfur and phenolic compounds. It has been found that administration of onion products to diabetic rats significantly reduced hyperglycemia. Furthermore, the role of nutritional factors in reproduction and subfertility is important. Research has shown that onion contains exogenous and endogenous antioxidants such as selenium, glutathione, vitamins A, B, and C, and flavonoids such as quercetin and isorhamnetin [3]. *Allium cepa* is used by Egyptian farmers since long time ago in poultry diets because they believed that has a beneficial effect on lowering the level of cholesterol in blood plasma and serum, protective effect against many diseases. Moreover, it has valuable nutrients such as vitamins, minerals, essential amino acids and essential fatty acids [4].

Zinc is the second most abundant transition metal after iron which in the body, it exists as $Zn^{2+}$. Zinc as an essential trace element which have key role in almost all body function e.g. immune system, growth, protein and DNA synthesis and reproduction [5]. Natural foods are generally believed to be safer, healthier and less hazardous than foods containing artificial additives. Onion is used as phytogenic feed additive alternative to chemical growth promoters. Previously it is reported that fresh onion stimulates blood circulation, improve immune response and have antibacterial effects due to its contents of pungent

substances [6]. In addition, onion is rich in sulfur containing compounds mainly in the form of cystine derivatives, viz. S-alkyl cystine sulfoxides which are decomposed the enzyme allinase into a variety of volatile compounds such as thiosulfinates and polysulfides during extraction. These compounds possess antidiabetic, antibiotic, hypocholesterolaemic, fibrinolytic, and various other biological effects. In addition to volatile substances in alliums, there are nonvolatile sulfur-containing peptides and proteins which have been shown to have potential health benefits [7]. However, it is reported that addition of either garlic or onion powders to the olive oil diet reduced liver Fe and tibial Ca and Na levels but no change in tibial Zn level. Likewise, liver of rats fed on olive oil diet with onion powder has noticeable elevation in Zn, Mg and P and reduction in Fe and K levels [8]. Therefore, the purpose of the present study was to examine the influence of oral administration of fresh onion juice compared to Zn sulfate on serum Fe and Mg levels in rat.

## MATERIALS AND METHODS

### Animals

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 experimental groups (each include 3 groups and 6 replicate). The rats kept individually in stainless steel wire-bottomed cages, resided under laboratory conditions at temperature 23.1-25.8°C and the humidity 55-60%, 12 h lighting period in accordance to European community suggestions for laboratory animals. All animals offered fresh water and *ad libitum* access to chow pellets (Azarbayjan Co. Iran).

### Plant material

Fresh onion was obtained from Ilkhchi-Tabriz, East Azarbayjan province, Iran. *Allium Cepa. Linn* identified at division of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Iran.

### Analysis of onion juice

The flavonoid components of onion juice were determined by Shinoda test [9] at Tehran University of Medical Sciences. The chief flavonoid component in onion is Quercetin and determined using qualitative thin-layer chromatography (TLC). 10 mL of fresh onion juice dried in a vacuum then the resulting residue dissolved in 1 mL of methanol. Methanolic solution (20 mL) was spotted on a silica gel plate (10×20 cm, silica gel 60 GF254, Merck, Darmstadt, Germany) by EtOAc/MeOH (80:20) solvent system. Quercetin as vehicle purchased from Sigma chemical Co. (St. Louis, MO, USA). Then after developing and drying, 2 % $AlCl_3$ solution in methanol is used to spray TLC plate. To recognize quercetin in the onion samples yellow spot caused by quercetin was the identification factor at RF=0.6. Quercetin was separated via preparative TLC on silica gel and LIAISON analyzer used to determine quantity of quercetin in sample. Quercetin compared to a pure quercetin standard curve in 370 nm. The quercetin in experimental fresh onion samples was 11.2 mg per100 g.

### Experimental procedure

Onion juice (1 or 2 cc) provided to rats on a daily basis as gavages (gastro-oral). Zinc sulfate purchased from Merk (© Merck KGaA, Darmstadt, Germany) and 15 and 30 mg/kg was dissolved in water gavage to rats. Doses were calculated based on our previous and pilot studies [3, 10-14].

At first week of experiment, in order to adaptation to experimental condition, all groups received basal then groups were divided as follows:

Groups 1: basal diet (as the vehicle control),
Groups 2: basal diet + 1cc fresh onion juice,
Groups 3: basal diet + 2cc fresh onion juice,
Groups 4: basal diet + 15 mg/kg zinc sulfate complement,
Groups 5: basal diet + 30 mg/kg zinc sulfate complement,
Groups 6: basal diet + 1cc fresh onion juice + 15 mg/kg zinc sulfate complement,
Groups 7: basal diet + 1cc fresh onion juice + 30 mg/kg zinc sulfate complement,
Groups 8: basal diet + 2cc fresh onion juice + 15 mg/kg zinc sulfate complement,
Groups 9: basal diet + 2cc fresh onion juice + 30 mg/kg zinc sulfate complement.

All animals received treatments as gavage once daily and treated until 4 weeks. All Onion juice was obtained through a fruit juice before the experiments [3, 13].

### Biochemical assays

At the end study, 12 hours starvation given to animals and six rats per treatment were selected randomly from each group. Blood samples were taken by the tail tip [15], centrifuged at 4°C for 10 min at 250×g and the serum obtained was stored at -20°C until assayed. Serum Mg and Fe concentration determined using colorimetric assay using commercial kit (Pars Azmoon Co., Tehran, Iran). All biochemical procedures have done using automatic biochemical analyzer (Mindray-BS-200, Germany). 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 protocols for animal experiment were approved by the institutional animal ethical committee.

**Statistical analysis**

This study was performed as a factorial 3×3 experiment (3 level of fresh onion juice and 3 level 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 [16]. When significant difference among the means was found, means were separated using Duncan's multiple range tests. $P \leq 0.05$ considered significant difference between groups. The result of the Analysis of variance according to the model is

$Yijk = \mu + \alpha i + \beta j + (a\ \beta)\ i\ j + eijk$

Where,

Yijk = All dependent variable

$\mu$ = Overall mean

$\alpha i$ = The fixed effect of onion levels (i = 1, 2, 3)

$\beta j$ = The fixed effect of zinc sulfate levels (j = 1, 2, 3)

eijk = The effect of experimental error

## RESULTS

Results of administration different levels of fresh onion juice on serum Fe and Mg compared with Zn sulfate supplementation in the rats is presented in table 1. According to the results, administration of sole fresh onion juice (1 or 2 cc) had no significant effects on serum Fe in 4 weeks treated rats (P>0.05). Also, Zn sulfate (15 or 30 mg/Kg) was not able to change serum Fe levels compared to control groups (P>0.05). Furthermore, co-administration of different levels of fresh onion juice (1 or 2 cc) and Zn sulfate (15 or 30 mg/Kg) had no prominent effects of serum Fe levels compared to the control group after 4 weeks treatment in rat (P>0.05).

Then we studied effects of onion juice and Zn sulfate on serum Mg concentrations. As seen in table 1, no significant difference observed on serum Mg levels in rat received different levels of fresh onion juice (P>0.05). In addition, sole Zn sulfate gavage was not able to cause significant fluctuations on serum Mg compared to control group (P>0.05). Moreover, simultaneous fresh onion juice and Zn sulfate administration had no significant effect on serum Mg concentrations after 4 weeks in rat (P>0.05).

**Table 1.** Effects of different levels of fresh Onion (*Allium Cepa. Linn*) juice on serum Fe and Mg compared with Zn sulfate supplementation in the rats.

| | | Fe (µg/dl) | Mg (mg/dL) |
|---|---|---|---|
| **Onion (cc)** | | | |
| 0 (control) | | 186.64 | 2.14 |
| 1 | | 203.55 | 1.91 |
| 2 | | 201.33 | 2.25 |
| P-value | | 0.67 | 0.11 |
| SEM | | 15.57 | 0.12 |
| **Zn sulfate supplementation (mg / kg)** | | | |
| 0 (control) | | 195.00 | 2.12 |
| 15 | | 181.90 | 2.10 |
| 30 | | 214.00 | 2.17 |
| *P-value* | | 0.43 | 0.77 |
| *SEM* | | 15.57 | 0.12 |
| **Combination administration** | | | |
| **Onion (cc)** | **Zn sulfate supplementation (mg / kg)** | | |
| 0 | 0 | 191.40 | 2.34 |
| | 15 | 164.00 | 2.06 |
| | 30 | 201.33 | 2.23 |
| 1 | 0 | 207.66 | 1.77 |
| | 15 | 176.00 | 2.00 |
| | 30 | 228.00 | 1.95 |
| 2 | 0 | 188.33 | 2.10 |
| | 15 | 207.66 | 2.26 |
| | 30 | 108.00 | 2.40 |
| *P-value* | | 0.85 | 0.71 |
| *SEM* | | 26.93 | 0.21 |

Zn: Zinc, Fe: Iron, Mg: Magnesium. SEM: standard error mean.

## DISCUSSION

To our knowledge this is a first study to investigate co-administration of fresh onion juice and Zn sulfate on serum Fe and Mg concentrations in rat. There are many trace elements in the body that directly or indirectly participate in metabolism and play an essential role in it. More than 25% of the enzymes in the body require metals for activation and to function properly in metabolism. Of these metals, iron, magnesium, and zinc have been identified as helping to improve cognitive functions in school children [17]. Iron is one of the important mineral elements necessary for the effective metabolism of the mammalian body. Although it is present in very small amounts in the body, Fe plays an important role in many metabolic processes. Iron plays a vital role in oxygen transport and energy production. The deficiency of iron continues to be a widespread condition affecting millions of people throughout the world [18]. In this study, administration of fresh onion juice or Zn had no effect of serum Fe level. It seems the administrated level of onion or Zn sulfate were not sufficient to impress its beneficial effects. Also, this result can use as a base dosage for further studies on effects of fresh onion juice and Zn sulfate on serum Fe levels in rat. Zinc ions are co-factors of many liver enzymes such as fructose-1, 6-bisphosphatase, alkaline phosphatase, and ethyl alcohol dehydrogenase. A large amount of zinc has been found in the cerebrum hippocampus area and profoundly affects memory formation; zinc status could therefore influence human memory [18]. Previously it is indicate that Zn acts as antioxidant reducing the cell membrane damage due to radicals, which in turn according to Powell, alters the immunological status of the animal. It has been suggested that Zn increases the synthesis of metallothionein, a cystine-rich protein, which acts as a free radical scavenger. In the other study showed that growth-furthering effects of Zn have been ascribed to effects on intestinal microflora [19]. In this study we were not able to find a research to compare our results.

The obtained results clearly indicated that administration of fresh onion juice or Zn had no effect on serum Mg levels in 4 weeks treated rats. The role of fresh onion juice or Zn sulfate on magnesium has rarely been discussed. Magnesium is responsible for the activation of more than 300 enzymes in the body. It assists to maintain muscle and nerve function in human body. Magnesium helps human heart rhythm to function at normal rate and supports a healthy immune system. Research indicates that Mg helps regulate blood sugar levels and helps blood pressure to function normally. The role of Mg according to the studies is loud and clear. It prevents and manages hypertension, cardiovascular disease, most importantly diabetes, and joint pains [20]. In this regards, it is reported adding garlic or onion (2% level) in the diet rich in mono or poly- unsaturated fatty acids elevate the levels of Mg in liver and tibia of rats, which contributes to the maintenance of the health status of the liver and bone [8]. Mineral bioavailability could be influenced by several factors in the diet such as inhibitors and promoters in meal and diet composition [21]. It seems the administrated level of onion or Zn sulfate were not sufficient to impress its beneficial effects on serum Mg. Also, this result can use as a base dosage for further studies on effects of fresh onion juice and Zn sulfate on serum Mg levels in rat. We recommend further researches need to clarify effective dosage of co-administration of onion juice and Zn sulfate. Additionally, merit studies are needed to distinguish their potential for clinical use in clinical trials.

### Conflicts of Interest

The authors declare that there are no conflicts of interest.

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