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EFFECTS OF ONION (*ALLIUM CEPA. LINN*) JUICE ON SERUM VALUES OF UREA, URIC ACID AND CREATININE COMPARED TO ZINC SULFATE SUPPLEMENTATION IN THE RATS

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ABSTRACT

The present study investigate the effects of fresh onion juice on serum Urea, Uric acid and Creatinine 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 urea, uric acid and creatinine levels (P>0.05).

Keywords: Onion, Glucose, Urea, Uric acid, Creatinine, Zn sulfate, Rat

INTRODUCTION

Onion (*Allium cepa L.*) is a member of the Alliaceae family and belongs to the genus Allium which consists of about 450 species. Besides onion and garlic, also the other members of Allium like leek (*Allium porrum L.*), shallot (*Allium ascalonicum L.*), wild garlic (*Allium ursinum L.*) and many others are known for their unique and typical taste. Allium plants are widely distributed over Europe, Asia and America, and they have been used for millennia as spices, vegetables and for the treatment of diseases **[1]**. They are a rich source of bioactive compounds which are the thiosulfinates and structurally related substances, flavonoids, sapogenins and saponins. Their biosynthetic pathways and metabolisms within the plant were mainly studied by chemical analysis and radiotracer experiments **[2]**.

Zinc is the second most abundant transition metal after iron which in 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 **[3]**. Urea is synthesized in the body of many organisms as part of the urea cycle, either from the oxidation of amino acids or from ammonia. In this cycle, amino groups donated by ammonia and L-aspartate are converted to urea, while L-ornithine, citrulline, L-argininosuccinate, and L-arginine act as intermediates. Urea production occurs in the liver and is regulated by N-acetylglutamate. Amino acids from ingested food that are not used for the synthesis of proteins and other biological substances are oxidized by the body, yielding urea and carbon dioxide, as an alternative source of energy. The oxidation pathway starts with the removal of the amino group by a transaminase, the amino group is then fed into the urea cycle. Ammonia (NH_3) is another common byproduct of the metabolism of nitrogenous compounds. Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen. It forms ions and salts known as urates and acid urates such as ammonium acid urate. Uric acid is a product of the metabolic breakdown of purine nucleotides. Creatinine is a metabolic by product of muscle metabolism (it is derived from creatine and phosphocreatine). For the majority of patients the muscle turnover varies little from day to day, and the serum creatinine is more or less constant. Creatinine is filtered

and excreted by the kidney. Serum creatinine is probably the most widely used indirect measure of glomerular filtration rate; it is easy and inexpensive to measure. There is little or no tubular reabsorption of creatinine **[4]**. Onion plays an important role in the Mediterranean diets for their dietary and medicinal properties. It has been suggested to be hypolipidemic, hypoglycemic, anticoagulant, antihypertensive, antimicrobial, anticancer, antitumor, hepatoprotective, immunomodulator and as an antidote for heavy metal poisoning. Onion possesses significant antioxidant effects because of their content of organo-sulfur compounds such as ajoene, alliine and allicin **[5]**. Antioxidant activity from a high intake of fruits and vegetables has been reported to prevent alteration of DNA by reactive oxygen species (ROS) and subsequent cancer development has been widely studied for their antioxidative effects **[6, 7]**. Reactive nitrogen species (RNS) give rise to, and act much like reactive oxygen species in causing oxidative damage to cellular proteins, tissues, and DNA **[8]**. Quercetin (main onion flavonoid) has been shown to reduce the level of peroxynitrate, an extremely powerful oxidant in the brain, by scavenging superoxide onion **[9]**. Based on previous researches the aim of this study was to evaluate Effects of fresh onion juice on serum values of urea, uric acid and creatinine compared to Zn sulfate supplementation in the rats.

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. The *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 **[10]** 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 **[4, 11-15]**.

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 juicer before the experiments **[11, 14]**.

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 **[16]**, centrifuged at 4°C for 10 min at 250×g and the serum obtained was stored at -20°C until assayed. Serum urea, uric acid and creatinine and 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 **[17]**. 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

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (a\ \beta)\ i\ j + e_{ijk}$$

Where,
Yijk = All dependent variable
μ = Overall mean
αi = The fixed effect of onion levels (i = 1, 2, 3)
βj = The fixed effect of zinc sulfate levels (j = 1, 2, 3)
eijk = The effect of experimental error

RESULTS

The result of administration different levels of fresh onion juice (1 or 2 cc) and Zn sulfate (15 or 30 mg/kg) is presented in **Table 1**. According to the data, sole drenching fresh onion juice had no significant effect on serum urea levels compared to control group in rat (P>0.05). Also, there was no significant effect on serum urea levels after 4 weeks single Zn sulfate application on serum urea levels when compared to control group in rat (P>0.05). Additionally, co-administration of fresh onion juice and Zn sulfate caused on significant effect on serum urea in 4 weeks treated rat in comparison to control group (P>0.05).

As seen from the data, single gavage of fresh onion juice had no effects on serum uric acid activity in 4 weeks treated rat compared to control group (P>0.05). Also, the same results observed after administration on Zn sulfate on serum uric acid in rat (P>0.05). Furthermore, co-administration of fresh onion juice and Zn sulfate had no significant effects on uric acid in rat (P>0.05).

According to data, single fresh onion juice at levels of 1 or 2 cc had no significant effects on serum creatinine in 4 weeks treated rat compared to control group (P>0.05). Furthermore, nor Zn sulfate nor simultaneous administration of different levels of fresh onion juice (1 or 2 cc) plus Zn sulfate (15 or 30 mg/kg) had no significant effects on serum creatinine after 4 weeks treatment compared to control group in rat (P>0.05).

DISCUSSION

Elevations of biochemical parameters such as plasma or serum urea, uric acid and creatinine are considered reliable for investigating drug-induced nephrotoxicity in animals and man. Elevated levels of uric acid and creatinine have been reported as a constant finding in lead toxicity creatinine **[4]**. Previously it is reported that garlic extract decreased the serum urea and

creatinine levels in diabetic rats. Elevation of the serum urea and creatinine, as significant markers, are related to renal dysfunction in diabetic hyperglycemia **[18]**. In our previous study administration of different levels of garlic juice (60 or 120 mg /kg) had no significant effects on serum urea, uric acid and creatinine concentration in 4 weeks treated rats **[4]**. The result of the current study was similar to our prior findings. It seems the administrated fresh onion juice levels were not sufficient to diminish urea, uric acid and creatinine concentration in rat. Also, the obtained results can use as reference range for further studies.

Creatinine is a waste product in the blood created by the normal breakdown of muscle during activity. Healthy kidneys take creatinine out of the blood and put it in the urine to leave the body. Creatinine builds up in the blood in kidney disease. The significant increase in creatinine and urea in the present study may be attributed to renal damage due to administration of carbonated meat. Dietary onions partailly reversed the abnormalities in blood urea and creatinine in streptozotocin induced diabetes mellitus rats. The significant decrease in creatinine and uric acid in the present study may be due to the higher antioxidants activities of leek. The non-nutrient constituents influence biotransformation enzymes involved in activation and detoxification of xenobiotic compounds **[19]**.

Zinc biology is a rapidly developing field, and recent research reveals zinc's strategic role in most organ systems. Physiologically, zinc is vital for growth and development and for healthy functioning of many body systems, encompassing insulin storage and release, cognition, cell membrane integrity, sexual maturation and reproduction, dark vision adaptation, olfactory and gustatory activity, thyroid function, blood clotting, taste acuity and for a variety of host immune defenses, among others. Clinical manifestations of zinc deficiency in humans include growth retardation, male hypogonadism, mental lethargy, abnormal neurosensory changes, delayed wound healing, skin changes, poor appetite, oligospermia, anorexia, weight loss, and susceptibility to infection **[20]**.

Thus, the elevated levels of circulating uric acid levels may be an indicator that the body is trying to protect itself from the deleterious effects of free radicals by increasing the products of endogenous antioxidants, such as uric acid. Interestingly, uric acid prevents oxidative modification of endothelial enzymes and preserves the ability of endothelium to mediate vascular dilatation in the face of oxidative stress **[21]**. Zinc is also responsible for the process of stabilizing cellular membranes, as well as

for inhibiting the processes of lipid peroxidation. The possible mechanism which revealed for hepato-protective role of Zn is that this metal is considered to be bound to or oxidized by endogenous sulfhydryls mediating cellular responses to injury by producing cytoprotection **[22]**. In case of Zn, it is evidence which Zn impresses its anti-oxidant mechanism via two pathway Chronic and Acute effects. In the first pathway, Zn stimulates anti-oxidant compounds activity such as metallothioneins **[23, 24]**. In case of our results, there was no previous study on administration on onion and Zn sulfate on serum urea, uric acid and creatinine. Therefore, we were not able to compare our results with. In agreement with previous studies our results confirmed the potent antioxidant role of onion in the rat. We think quercetin as chief flavonoid in the onion might decreases semen urea, uric acid and creatinine levels by altering ROS production in testes but the direct mechanism is still unknown. It seems, this dosage can be used as a base dosage for further researches. 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 report no conflicts of interest.

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Table 1: Effects Of Different Levels Of Fresh Onion (*Allium Cepa. Linn*) Juice On Serum Values Of Glucose, Urea, Urease And Creatinine Compared With Zn Sulfate Supplementation In The Rats

		Urea (mg/dl)	U-A (mg/dl)	Creatinine (mg/dl)
Onion (cc)				
0 (control)		**46.88**	**1.90**	**0.53**
1		**47.98**	**1.38**	**0.57**
2		**38.70**	**1.71**	**0.55**
P-value		**0.43**	**0.29**	**0.93**
SEM		**5.37**	**0.22**	**0.07**
Zn sulfate supplementation (mg /kg)				
0 (control)		**42.07**	**1.71**	**0.54**
15		**48.10**	**1.51**	**0.56**
30		**44.80**	**1.76**	**0.54**
P-value		**0.75**	**0.71**	**0.99**
SEM		**5.37**	**0.22**	**0.07**
Combination administration				
Onion (cc)	**Zn sulfate supplementation (mg /kg)**			
0	**0**	**41.60**	**2.04**	**0.47**
	15	**55.53**	**1.37**	**0.54**
	30	**47.53**	**2.20**	**0.61**
1	**0**	**41.37**	**1.40**	**0.61**
	15	**49.60**	**1.30**	**0.62**
	30	**51.32**	**1.45**	**0.49**
2	**0**	**43.57**	**1.47**	**0.58**
	15	**38.67**	**1.93**	**0.53**
	30	**33.87**	**1.71**	**0.54**
P-value		**0.79**	**0.64**	**0.79**
SEM		**9.29**	**0.38**	**0.12**

Zn: Zinc, U-A: Uric Acid, Mg: Magnesium. SEM: Standard Error Mean