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**EFFECTS OF ONION (*ALLIUM CEPA. LINN*) JUICE ON SERUM VALUES OF
TOTAL PROTEIN, ALBUMIN AND GLOBULIN COMPARED WITH ZINC
SULFATE IN MALE RATS**

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ABSTRACT

The aim of the current study was to investigate effects of fresh onion juice on serum total protein (TP), albumin (Alb) and globulin (Glb) compared to zinc (Zn) sulfate in the rats. One hundred and sixty-two male rats randomly distributed into 9 experimental groups (each include 3 groups and 6 replicate). Group 1 served as control. In group 2, rat received 1cc fresh onion juice. In group 3, 2cc fresh onion juice was rats offered to rat. Group 4, gavage 15 mg/kg zinc (Zn) sulfate. In group 5, rats received with 30 mg/kg Zn sulfate. In group 6, 1cc fresh onion juice + 15 mg/kg Zn sulfate were offered. In group 7, 1cc fresh onion juice + 30 mg/kg Zn sulfate provided to rats. In group 8, animals consumed 2cc fresh onion juice + 15 mg/kg Zn sulfate. Group 9, provided with 2cc fresh onion juice + 30 mg/kg Zn sulfate. All animals received treatments as gavage once daily and had *ad libitum* access to chow pellets and fresh water. After 4 weeks, blood samples were obtained and serum TP, Alb and Glb activity determined. According to the results, there was no significant effect on serum TP, Alb and Glb post-treatment by onion or Zn ($P>0.05$).

Keywords: Onion juice, Total protein, Albumin, Globulin, Zinc, Rat

INTRODUCTION

Onion "*Allium cepa L*" is cultivated widely that has a beneficial effect on lowering the level of cholesterol in blood plasma and long time ago in diets because they believed serum have protective effect against many

diseases. Moreover, both have valuable nutrients such as vitamins, minerals, essential amino acids and essential fatty acids. Natural foods are generally believed to be safer more healthy and less hazardous than foods containing artificial additives. Both onion and garlic are used as phyto-genic feed additive alternative to chemical growth promoters [1]. Previously it is emphasized that fresh onion and/or garlic stimulate blood circulation, improve immune response and have antibacterial effects due to its contents of pungent substances [2]. Onion is used as a traditional remedy in the treatment of a variety of disorders. The pharmacological evidence for the use of onion as an anti-asthmatic, anti-hypertensive, anti-hyperglycemic, anti-hyperlipidemic and anti-tumor agent was reported [3]. 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, alliin and allicin [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]. Antioxidants such as flavonoids, polyphenols, vitamin C and E and carotenoids have been reported to protect the body system against reactive oxygen species. In the search for antioxidant compounds, various efforts are now concentrated on much herbal plant extracts because of their potential to induce antioxidant effects [6]. Accumulating evidence has demonstrated that overproduction of reactive oxygen species (ROS) plays a key role in the etiology of hepatocarcinoma. ROS could result in oxidative damage of DNA, which facilitating the formation of hepatocellular carcinomas [7].

Total protein content provides some information regarding a patient's general status; more clinically useful data are obtained from fractionating the total protein. Albumin makes up more than half of the total protein present in serum. Approximately 30 to 40% of the body's total albumin pool is found in the intravascular compartment. The liver produces albumin at less than half of its capacity. The primary factors affecting albumin synthesis include protein and amino acid nutrition, colloidal osmotic pressure, the action of certain

hormones, and disease states. Fasting or a protein deficient diet causes a decrease in albumin synthesis as long as the deficiency state is maintained. In the normal individual, the liver increases albumin synthesis in response to the increased availability of amino acids provided by the portal blood following each protein-containing meal. Globulin is positive acute phase proteins and its levels increase during many disease and parasites infections. Therefore, the changes of plasma total protein concentration and Alb:Glb ratio is mainly depended to variation of albumin and globulin concentration [8]. The globulin fraction includes hundreds of serum proteins including carrier proteins, enzymes, complement, and immunoglobulins. Most of these are synthesized in the liver, although the immunoglobulins are synthesized by plasma cells. In our previous study, different levels of garlic aqueous extract (60 and 120 mg /kg) had no significant effect on serum TP, Alb and Glb levels on CrCl₃-exposed male rats [9]. Based on previous studies the objectives of this work were to investigate the effects of drenching fresh onion juice on Serum Values of TP, Alb and Glb compared with Zinc sulfate in Male 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 $\text{RF}=0.6$. 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 per 100 g.

Experimental Procedure

Onion juice (1 or 2 cc) provided to rats on a daily basis as gavages (gastro-oral). Zinc sulfate purchased from Merck (© 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 [11-16].

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, 15].

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, centrifuged at 4°C for 10 min at $250\times g$ and the serum obtained was stored at -20°C until assayed. Serum TP, Alb and Glb 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 \pm 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. $P \leq 0.05$ considered significant difference between groups. The result of the Analysis of variance according to the model is

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

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 obtained results in this study are illustrated in **Table 1**. According to the data, administration of sole fresh juice (1 or 2 cc) had no significant effect on serum TP levels in rat compared to control group ($P > 0.05$). In addition, single Zn sulfate supplementation (15 or 30mg /kg) had no significant effect on TP in rat in comparison to control group ($P > 0.05$). Also, there was no significant change on serum TP after co-administration of fresh onion juice and Zn sulfate in rat compared to control group ($P > 0.05$).

Based on the results, single administration of different levels of fresh onion juice had no significant effect on serum Alb levels after 4 weeks in rat ($P > 0.05$). Moreover, there was no significant change in serum Alb in rats gavage different levels of Zn

sulfate (15 or 30mg /kg) compared to control group ($P > 0.05$). As well, co-administration of fresh onion juice and Zn sulfate had no significant effect on serum Alb compared to control group ($P > 0.05$) (**Table 1**).

As seen in **Table 1**, no significant alteration observed in serum Glb levels in rat received 1 or 2 cc fresh onion juice after 4 weeks compared to control group ($P > 0.05$). Also, 15 or 30 mg /kg Zn sulfate was not able to cause significant alteration on serum Glb levels in rat ($P > 0.05$). According to the result, simultaneous administration of fresh onion juice (1 or 2cc) and Zn sulfate (15 or 30mg /kg) had no significant effect on serum Glb in 4 weeks treated rats ($P > 0.05$).

DISCUSSION

For centuries consumption of fruits and vegetables has been attributed to beneficial health effects. Ames and Gold [17] 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 [18]. Free radical formation of reactive oxygen species (ROS) and subsequent oxidative stress have been correlated to many human disorders including those of the kidney, eye, lung,

liver, nervous system, heart and cardiovascular system [19].

Onions have received considerable attention for their healthful, functional benefits. Phytochemicals in onions include the organosulfur compounds such as cepaenes and thiosulfinates [20], the large class of flavonoids including quercetin and kaempferol [21], and pigments such as anthocyanins found in red onions and red wine [22]. Potential benefits of onion consumption to human health are still under investigation. Promising results have been obtained from epidemiological studies, in vivo research, and numerous in vitro investigations. Some in vivo research contradicts in vitro findings, while other researchers suggest a possible different mechanism of benefit. As with most unproven theories, there will be both supporting and dissenting evidence. Therefore, some findings are accepted while others need more research to support or refute the available evidence. The volume of scientific research concerning healthful benefits of onion phytochemicals continues to increase as more attention is focused on food in prevention and treatment of human diseases. Rats fed 2g/kg dry onion for six days while feeding on an atherogenic diet showed significant reductions in both serum cholesterol and triglyceride levels as compared to those only eating the

atherogenic diet [23]. Likewise, students fed a high fat diet one day, and the same diet plus 100g onions another showed a significant decrease in serum triglycerides, but not cholesterol, from the onion supplemented diet. Investigators from both studies showed a possible inhibitory benefit of onion consumption on atherosclerosis formation. Quercetin has been shown to not only directly inhibit the lipoxygenase enzyme, but to also suppress consumption of alpha-tocopherol and to preserve human serum paraoxonase (PON 1), both potent antioxidants against lipid peroxidation [24]. Metal chelation involves formation of a complex with the flavonoid and prevention of catalytic radical production, whereas free radical scavenging activities relate to the flavonoid donating a hydrogen atom and creating a more stable radical [25]. Quercetin is one of the well-studied flavonoids and is particularly high in onions. Quercetin is thought to be protective against coronary heart disease, stroke, and certain cancers. But most flavonoids, including quercetin, have very low oral bioavailability. Thus their absorption from food may be limited. Walle *et al.*, [26] examined radiolabeled quercetin that had been administered orally and intravenously (IV) to normal, healthy volunteers. Total recovery of the labeled carbon in the quercetin was very low. These workers

found that a large of the quercetin dose was recovered as carbon dioxide in the expired air from volunteers after both oral and IV doses. Thus, much more work must be done to determine the metabolism of quercetin in vivo in order to estimate its potential for human health.

Proteins are the most abundant compounds in serum. Amino acids are the building blocks of all proteins. In turn proteins are the building blocks of all cells and body tissues. They are the basic components of enzymes, many hormones, antibodies and clotting agents [27]. Proteins act as transport substances for hormones, vitamins, minerals, lipids and other materials. In addition, proteins help balance the osmotic pressure of the blood and tissue. Osmotic pressure is part of what keeps water inside a particular compartment of your body [28, 29]. Proteins play a major role in maintaining the delicate acid-alkaline balance of your blood. Finally, serum proteins serve as a reserve source of energy for your tissues and muscle when you are not ingesting an adequate amount. The major measured serum proteins are divided into two groups, albumin and globulins. There are four major types of globulins, each with specific properties and actions. A typical blood panel will provide four different measurements - the total protein, albumin, globulins, and the albumin

globulin ratio [27]. In case of our results, there was no previous study on administration on onion and Zn sulfate on serum TP, Alb and Glb. 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 TP, Alb and Glb 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 declare that there are no conflicts of interest.

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Table 1: Effects of Different Levels of Fresh Onion (<i>Allium Cepa. Linn</i>) Juice on Serum Values of Total Protein, Albumin and Globulin Compared with Zn Sulfate Supplementation in the Rats				
		TP (gr/dL)	Alb (gr/dL)	Glb (gr/dL)
Onion (cc)				
0 (control)		7.12	3.30	3.82
1		7.11	3.14	3.97
2		7.19	3.18	4.01
P-value		0.91	0.48	0.51
SEM		0.16	0.39	.025
Zn sulfate supplementation (mg /kg)				
0 (control)		7.16	3.23	3.39
15		7.06	3.23	3.83
30		7.21	3.16	4.05
P-value		0.91	0.48	0.53
SEM		0.16	0.39	0.17
Combination administration				
Onion (cc)	Zn sulfate supplementation (mg /kg)			
0	0	7.33	3.60	3.73
	15	6.60	3.00	3.60
	30	7.38	3.25	4.13
1	0	7.11	3.00	4.11
	15	7.35	3.40	3.95
	30	7.10	3.00	4.00
2	0	7.00	3.00	3.92
	15	7.17	3.25	3.92
	30	7.18	3.33	3.85
P-value		0.28	0.80	0.36
SEM		0.1	0.68	0.29
Zn: Zinc, TP: Total Protein, Alb: Albumin, Glb: Globulin, Mg: Magnesium. SEM: Standard Error Mean				