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Effect of Garlic (*Allium sativum*) Aqueous Extract on serum values of Urea, Uric-Acid and Creatinine compared with Chromium Chloride in Male Rats

Jamshid Ghiasi Ghalehkandi[1]*, Yahya Ebrahimnezhad[1], Ramin Salamatdout Nobar[1]

[1] *Department of Animal Science, Faculty of Agriculture, Shabestar Branch, Islamic Azad University, Shabestar, East Azerbaijan, 5381637181, Iran*

ABSTRACT

*Garlic (Allium sativum) is a biennial herb of the family Liliaceae. It contains various active components like allicin and its derivatives S-allyl cysteine, diallyldisulfide, diallyltrisulfide which increase the sulfhydryl groups available to form soluble complexes with lead and pairing the essential sulfidryl groups of enzymes and protein thereby preventing more internal toxicity. In this experiment, one 162 mature male rats (250 gr on the average) were acquired from Razi Serum – producing Institute of Karaj-Iran and transferred to keeping place. This design is performed as a factorial experiment 3*3 (3 level of Garlic Aqueous Extract (GAE) extract and 3 level of Chromium Chloride ($CrCl_3$) supplement) in the form of totally random design with 9 groups per 3 replications each containing 6 rats. At the end of fourth week, after 12 hours starvation, six rats per treatment were selected randomly from every treatment and their blood sampling was collected for biochemical traits, then serum concentration of creatinine, urea and uric acid were determined. It revealed that garlic aqueous extract results in decrease urea and creatinine and increase in uric acid but these changes were not significant. Also, $CrCl_3$ supplementation as single use yields to decrease urea and uric acid ($P>0.05$) and increase creatinine ($P<0.05$). Data showed that combinative use of garlic and CrCl3 supplementation yields to decrease in urea when garlic was 60 mg/kg and $CrCl_3$ was 4 mg/kg also when garlic was 120 mg/kg and $CrCl_3$ was 4 mg/kg but this decrease was not significant ($P>0.05$). Combinative use of garlic and $CrCl_3$ supplementation yields to increase in creatinine values in all doses and uric acid when garlic was 60 mg/kg and $CrCl_3$ was 4 mg/kg ($P\leq0.05$).*

Keywords: *Allium sativum*, Aqueous Extract, Urea, Uric-Acid, Creatinine, Chromium Chloride, Rats.

INTRODUCTION

A member of the *Liliaceae* family, garlic (*Allium sativum*) is a cultivated food highly regarded throughout the world. Originally from Central Asia, garlic is one of the earliest of cultivated plants. The Ebers Codex and Egyptian medical papyrus dating to about 1550 B.C.E. mentions garlic as an effective remedy for a variety of ailments. Early men of medicine such as Hippocrates, Pliny and Aristotle espoused a number of therapeutic uses for this botanical [28]. Today it is commonly used in many cultures as a seasoning or spice. According to the US Food and Drug Administration survey of 900 people, garlic stands as the second most utilized supplement (behind Echinacea), with almost 17% of the population using garlic supplement in the preceding 12 months [35]. Most of the garlic eaten today comes from China, South Korea, India, Spain, and the United States. In addition to its reputation as a healthy food, garlic has shown anti-viral, anti-bacterial, antifungals and antioxidant capacities. Additionally, anti-

atherosclerotic and anti-cancer properties have also been demonstrated. The genus Allium includes garlic, scallions, onions, chives, and leeks. These contain the sulfur compounds which are medicinally active.

The majority of reported medicinal effects of this botanical appear to come from the sulfur containing compounds, high trace mineral content, and enzymes. Most of the sulfur found in whole garlic cloves is of two types found in equal quantities: the S-alkylcysteine sulfoxides and the γ-glutamyl-S-alkylcysteines. The most abundant sulfur compound in garlic is alliin (S-allylcysteine sulfoxide), which is present at 10 mg/g in fresh garlic or 30 mg/g dry matter [23]. Recent studies from Korea have further elucidated novel sulfur containing nitrogenous compounds responsible for the greening process of crushed or bruised garlic. These compounds are not released when the garlic is finely peeled and have been found to differ significantly from other green plant pigments [24]. It is clear that even with a plant medicine as well characterized as garlic, there is still much to be learned.

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. Urea is found dissolved in blood (in the reference range of 2.5 to 6.7 mmol/L) and is excreted by the kidney as a component of urine. In addition, a small amount of urea is excreted (along with sodium chloride and water) in sweat. 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 [32]. 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. Ammonia is smaller, more volatile and more mobile than urea. If allowed to accumulate, ammonia would raise the pH in cells to toxic levels. Therefore many organisms convert ammonia to urea, even though this synthesis has a net energy cost. Being practically neutral and highly soluble in water, urea is a safe vehicle for the body to transport and excrete excess nitrogen. In water, the amine groups undergo slow displacement by water molecules, producing ammonia and carbonate anion. For this reason, old, stale urine has a stronger odor than fresh urine [26].

Uric acid is a heterocyclic compound of carbon, nitrogen, oxygen, and hydrogen with the formula $C_5H_4N_4O_3$. 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. High blood concentrations of uric acid can lead to a type of arthritis known as gout. The chemical is associated with other medical conditions including diabetes and the formation of ammonium acid urate kidney stones [26].

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. If the filtering of the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance ($CrCl_3$), which reflects the glomerular filtration rate (GFR) [13].

The GFR is clinically important because it is a measurement of renal function. However, in cases of severe renal dysfunction, the creatinine clearance rate will be "overestimated" because active secretion of creatinine from the proximal tubule will account for a larger fraction of the total creatinine cleared [13].

Previous studies have shown that organic forms of chromium, unlike mineral forms, are very toxic in the body due to higher absorption (20 to 30 times). Therefore, organic form of chromium is very toxic [36]. The body requirement to it is not accurately determined yet. But researches showed that in cases with tension (thermal and nutritional) or infection, the need for this element increases due to increased excretion of that through urine [36]. The chromium has important role in the body metabolic and heat stress; so, nowadays it is used in food as supplementation. The main objective of present study was to evaluation of effect of Garlic (*Allium sativum*) aqueous extract on serum values of urea, uric acid and creatinine compared with chromium chloride in male rats.

MATERIALS AND METHODS

In this experiment, 162 mature male rats (250 gr on the average) were acquired from Razi Serum – producing Institute of Karaj-Iran and transferred to keeping place. This design is performed as a factorial experiment 3*3 (3 level of GAE extract and 3 level of $CrCl_3$ supplement) in the form of totally random design with 9 groups per 3 replications each containing 6 rat. All of keeping cages were disinfected before performing the experiment. All of groups were kept in 12-hour light and 12-hour darkness conditions with 25-30. Temperature and free access to water and food in metal cages placed in animal husbandry of veterinary faculty of Islamic Azad University, Tabriz Branch.

Preparation of garlic extract (GAE) and CrCl3 supplement

Fresh garlic aqueous was used in this experiment, and garlic aqueous extract was obtained through soxhlet apparatus in combination with deionized distilled water within 6 hours in two successive days with temperature of 30 (to prevent elements and materials of garlic aqueous from decomposition). Then, the extract was placed in incubator in order to be concentrated. Certain concentrations of garlic aqueous extract were dissolved in pure water and became reachable by rat on a daily basis. $CrCl_3$ supplement was acquired (Merck-Germany) and after measuring certain rate by digital scale was given to rat on a daily basis. It should be mentioned that onion extract was give as gavage (gastro – oral) and $CrCl_3$ complement was dissolved in water in certain amount and it was added to feed after steeping and powdering of pellets, then the feed was mixed , ground and dried, and obtained pellets was given to animal. Moreover, during the first week of experiment, all groups consumed basal diet in order to adapt with breeding environment conditions; then basal diet, basal diet + 60 mg/rat/day fresh GAE, basal diet + 120 mg/rat/day fresh GAE, basal diet + 4 mg/ kg diet $CrCl_3$, basal diet + 8 mg/ kg diet $CrCl_3$, basal diet + 60 mg/ rat/day fresh GAE+ 4 mg/ kg diet $CrCl_3$, basal diet + 60 mg/ rat/day fresh GAE+ 8 mg/ kg diet $CrCl_3$, basal diet + 120 mg/ rat/day fresh GAE+ 4 mg/ kg diet $CrCl_3$ and 120 mg/ rat/day fresh GAE+ 8 mg/ kg diet $CrCl_3$, respectively, were given to 1st group, 2nd group, 3rd group,4th group,5th group,6th group, 7th group, 8th group, and 9th group, within 4 weeks on a daily basis.

Determination of the biochemical traits

At the end of fourth week, after 12 hours starvation, six rats per treatment were selected randomly from every treatment and their blood sampling was collected for biochemical traits, then serum concentration of creatinine, urea and uric acid were determined.

Statistical analysis

Data were subjected to a one-way analysis of variance using the General Linear Models (GLM), and the statistical analysis system User's guide. 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
μ = Overall mean
αi = The fixed effect of GAE levels (i = 1, 2, 3)
βj = The fixed effect of $CrCl_3$ levels (j = 1, 2, 3)
eijk = The effect of experimental error

When significant difference among the means was found, means were separated using Duncan's multiple range tests.

RESULTS

As shown in table 1, it reveals that garlic aqueous extract results in decrease urea and creatinine and increase in uric acid but these changes is not significant. Also, $CrCl_3$ supplementation as single use yields to decrease urea and uric acid ($P>0.05$) and increase creatinine ($P<0.05$).

Data showed that combinative use of garlic and $CrCl_3$ supplementation yields to decrease in urea when garlic was 60 mg/kg and $CrCl_3$ was 4 mg/kg also when garlic was 120 mg/kg and $CrCl_3$ was 4 mg/kg but this decrease was not significant ($P>0.05$).

Combinative use of garlic and $CrCl_3$ supplementation yields to increase in creatinine values in all doses and uric acid when garlic was 60 mg/kg and CrCl3 was 4 mg/kg ($P \leq 0.05$).

Table 1: Comparison of data obtained from analyzing of serum values of urea (mg/dl), uric acid (mg/dl) and creatinine (mg/dl)

Garlic		**Urea**	**Creatinine**	**Uric acid**
0 mg /kg (control)		40.36	0.61	1.81
60 mg /kg		38.36	0.53	2.05
120 mg /kg		43.83	0.58	2.44
P-value		0.53	0.79	0.55
SEM		2.76	0.05	0.41
Supplementary CrCl3				
0 (control)		39.15	0.54	2.51[a]
4 mg /kg		38.72	0.59	1.94[ab]
8 mg /kg		43.52	0.60	1.59[b]
P-value		0.45	0.91	0.03
SEM		2.76	0.14	0.41
Interaction				
Garlic	**Supplementary** $CrCl_3$			
0 mg /kg	0 (control)	41.91	0.47	2.04
	4 mg /kg	39.28	0.55	1.73
	8 mg /kg	40.90	0.93	1.57
60 mg /kg	0 (control)	34.24	0.56	2.23
	4 mg /kg	39.30	0.62	2.28
	8 mg /kg	42.20	0.40	1.55
120 mg /kg	0 (control)	45.12	0.60	3.87
	4 mg /kg	36.83	0.59	1.80
	8 mg /kg	47.80	0.56	1.67
P-value		0.68	0.67	0.54
SEM		4.77	0.23	0.70

Dissimilar letters (a,b) show significant difference in column.

DISCUSSION AND CONCLUSION

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 [1]. Elevated levels of uric acid and creatinine have been reported as a constant finding in lead toxicity. The mechanism through which lead exposure raises the level of uric acid is unclear but is thought to be due to damaged renal tubules by lead [25]. Beyer et al. (1988) have reported degeneration of kidney and altered kidney function due to lead accumulation [8].

Garlic or its extract (GE) [14,20,22,31] and garlic compounds [10] showed antioxidant properties. The use of garlic gave protection against drug-induced cardiotoxicity [3,11,27], nervous system disorders [9,17], drug-induced neurotoxicity [30], modulation of immunity [16,29], improved feed conversion in poultry [15], hypoglycemic agent [34], antimicrobial [6], in gastric ulcers associated with *Helicobacter pylori* [2], anticancer [19], antifungal [12], antiprotozoal [37] and anthelmintic actions [5,33].

Bagheri et al., (2011) showed that reperfusion increased serum urea and fractional excretion of sodium levels, while it decreased urine potassium levels and creatinine clearance. However, garlic juice significantly decreased serum urea levels in the reperfusion + garlic group compared with the reperfusion group ($P < 0.001$). Preteatment with garlic juice also resulted in significant increase in urine potassium ($P = 0.03$) compared to reperfusion. Fractional excretion of sodium and creatinine clearance was also improved. On histological examination, rats pretreated with garlic juice had nearly normal morphology. They concluded that garlic juice significantly prevented renal reperfusion-induced functional and histological injuries which are compatible with our research results [7].

Hassan et al., (2009) investigated the protective role of garlic oil against $NaNO_2$-induced abnormalities in metabolic biochemical parameters and oxidative status in male albino rats. They reported that NaNO2 treatment for a period of three months induced a significant increase in serum levels of glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin, urea and creatinine as well as hepatic AST and ALT. However, significant decrease was recorded in liver ALP activity, glycogen content, and renal urea and creatinine levels. In parallel, a significant increase in lipid peroxidation, and a decrease in glutathione content and catalase activity were observed in the liver and the kidney. However, garlic oil supplementation showed a

remarkable amelioration of these abnormalities. Our data indicate that garlic is a phytoantioxidant with powerful chemopreventive properties against chemically-induced oxidative stress [18].

In one study by Jabbari et al., 2005 results indicated that although lipid profile, BUN, Cr, serum levels of cyclosporine and diastolic blood pressure did not change, Systolic blood pressure decreased from 138.2 to 132.8 mmHg (p=0.001) and Malondialdehyde (MDA) decreased from 2.4 to 1.7 nmol/ml (p=0.009) by swallowing route, Cholesterol decreased from 205.1 to 195.3 mg/dl (p=0.03), triglyceride decreased from 195.7 to 174.8 mg/dl (p=0.008), MDA decreased from 2.5 to 1.6 nmol/ml (p=0.001), systolic blood pressure decreased from 137.5 to 129.8 mmHg (p=0.001), diastolic blood pressure decreased from 84.6 to 77.6 mmHg (p=0.001) and Cr decreased from 1.51 to 1.44 mg/dl (p=0.03) by chewing route too, it is compatible with our research [21].

Zha et al., (2007) worked on the supplementation of chromium nanoparticles and they found that these significantly increased average daily gain, food efficiency, and lean body mass and decreased fat mass and body fat proportion and serum levels of glucose, urea nitrogen, triglyceride, and insulin. Chromium contents in liver, kidney, and hind leg muscle were increased significantly with the addition of CrNano in diet. They results indicate that chromium nanocomposite has higher efficacy on growth and body composition compared to the traditional chromium agents [38].

Contrary, Anderson et al., (1997) demonstrated that there is no statistically significant differences in body weight, organ weights or blood variables among all the groups tested at 11, 17 and 24 weeks. Blood variables measured were glucose, cholesterol, triglycerides, blood urea nitrogen, lactic acid dehydrogenase, transaminases, total protein and creatinine. Histological evaluation of the liver and kidney of control and animals fed 100 mg/kg Cr as Cr chloride or picolinate also did not show any detectable differences. Liver and kidney Cr concentrations increased linearly for both the Cr chloride and picolinate fed animals. They concluded that lack of toxicity of trivalent Cr, at levels that are on a per kg basis, several thousand times the upper limit of the estimated safe and adequate daily dietary intake for humans. Animals consuming the picolinate supplemented diets had several-fold higher Cr concentrations in both the liver and kidney than those fed Cr chloride. Based on our results, can conclude that garlic has ameliorative activity on urea, creatinine and uric acid which is attributed active component of garlic which is called allicin. Also, about chromium is due to its active components which is mentioned heavy metals in the article, but other researches need to approve it [4].

REFERENCES

[1] Adelman RD, Spangler WL, Beason F, Ishizaki G, Conzelman GM, *Journal of Antimicrobials and Chemotherapy*, **1981**, 7, 431-435.
[2] Adeniyi BA, Oluwole FS, Anyiam FM, *J. Boil. Sci.*, **2006**, 6, 521-526.
[3] Alkreathy H, Damanhouri ZA, Ahmed N, Slevin M, Ali SS, Osman AMM, *Food Chem. Toxicol*., **2010**, 48, 951-956.
[4] Anderson RA, Bryden NA, Polansky MM, *J Am Coll Nutr*., **1997**, 16(3), 273-9.
[5] Ayaz E, Turel I, Gul A, Yilmaz O, Recent Pat. *Antiinfect. Drug Discov*., **2008**, 3, 149-152.
[6] Bachrach G, Jamil A, Naor R, Tal G, Ludmer Z, Steinberg D, *J. Med. Food*, **2011**, 14(11), 1338-43.
[7] Bagheri F, Gol A, Dabiri S, Javadi A, *Iran J Kidney Dis*., **2011**, 5(3), 194-200.
[8] Beyer WN, Spann JW, Sileo L, Fronsen JC, *Toxicol*, **1988**, 17, 121.
[9] Chauhan NB, Sandoval J, Phytother. *Res*., **2007**, 21, 629-640.
[10] Chung LY, *J. Med. Food*, **2006**, 9, 205-213.
[11] Das RN, Poudel N, *Kathmandu Univ. Med. J*., **2006**, 4, 337-339.
[12] Davis SR, *Mycoses*, **2005**, 48, 95-100.
[13] Delanghe J, De Slypere JP, De Buyzere M, Robbrecht J, Wieme R, Vermeulen A, *Clin. Chem.*, **1989**, 35(8), 1802-3.
[14] Dillon SA, Burmi RS, Lowe GM, Billigton D, Rahman K, *Life Sci*., **2003**, 72, 1583-1594.
[15] Fadlalla IMT, Mohammed BH, Bakhiet AO, *Asian J. Poult. Sci.*, **2010**, 4, 182-189.
[16] Ghazanfari T, Hassan ZM, Khamesipour A, *J. Ethnopharmacol.*, **2006**, 103, 333-337.
[17] Gupta VB, Indi SS, Rao KS, *Phytother. Res.*, **2009**, 23, 111-115.
[18] Hassan HA, El-Agmy SM, Gaur RL, Fernando A, Raj MH, Ouhtit A, *Int. J. Biol. Sci*., **2009**, 5(3), 249-55.
[19] Huang YS, Xie N, Su Q, Su J, Huang C, Liao QJ, *Mol. Med. Rep*., **2011**, 4, 553-559.
[20] Imai J, Ide N, Nagae S, Moriguchi T, Matasuura H, Itakura Y, *Planta Med.*, **1994**, 60, 417-420.

[21] Jabbari A, Argani H, Ghorbanihaghjo A, Mahdavi R, *Lipids Health Dis.*, **2005**, 19, 4-11.
[22] Jeong YY, Park HJ, Cho YW, Kim EJ, Kim GT, Mun YJ, Lee JD, Shin JH, Sung NJ, Kang D, Han J, *Phytother Res.*, **2012**, 26(1), 18-25.
[23] Lawson LD, *American Chemical Society*, **1998**, 176-209.
[24] Lee EJ, Cho JE, Kim JH, Lee SK, *Food Chemistry*, **2007**, 101(4), 1677-1686.
[25] Loghman-Adham M, *Environ. Health*, **1997**, 105(9), 928-938.
[26] Marsh KL, Sims GK, Mulvaney RL, *Biol. Fert. Soil*, **2005**, 42, 137-145.
[27] Mukherjee S, Banerjee SK, Maulik M, Dinda AK, Talwar KK, Maulik SK, *BMC Pharmacol.*, **2003**, 3, 16-16.
[28] Murray M, The Encyclopedia of Healing Foods, *Atria Books*, **2005**, 201.
[29] Nya EJ, Austin B, *Fish Shellfish Immunol.*, **2011**, 30, 845-850.
[30] Perez-Severiano F, Rodriguez-Perez M, Pedraza-Chaverri J, Maldonado PD, Medina-Campos ON, Ortíz-Plata A, Sánchez-García A, Villeda-Hernández J, Galván-Arzate S, Aguilera P, Santamaría A, *Neurochem. Int.*, **2004**, 45, 1175-1183.
[31] Põldma P, Tõnutare T, Viitak A, Luik A, Moor U, *J. Agric. Food Chem.*, **2011**, 59, 5498-5503.
[32] Sakami W, Harrington H, *Annual Review of Biochemistry*, **1963**, 32(1), 355-98.
[33] Singh TU, Kumar D, Tandan SK, Mishra SK, *Exp. Parasitol.*, **2009**, 123, 302-308.
[34] Sukandar EY, Permana H, Adnyana IK, Sigit JI, Ilyas RA, Hasimun P, Mardiyah D, *Int. J. Pharmacol.*, **2010**, 6, 456-463.
[35] Timbo BB, Ross MP, McCarthy PV, Lin CT, *Am Diet Assoc.*, **2006**, 106(12), 1966-74.
[36] Underwood EJ, Suttle NF, *The mineral nutrition of livestock*, 3rd, CAB International, Wallingford, UK., **1999**, 517-518.
[37] Wabwoba BW, Anjili CO, Ngeiywa MM, Ngure PK, Kigondu EM, Ingonga J, Makwali J, *J. Vector Borne Dis.*, **2010**, 47, 160-167.
[38] Zha LY, Wang MQ, Xu ZR, Gu LY, *Biol Trace Elem Res.*, **2007**, 119(1), 42-50.