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# THE EFFECT OF AQUEOUS GARLIC EXTRACT AND CHROMIUM CHLORIDE COMPLEMENT ON TISSUE ANTI OXIDANT SYSTEM OF MALE RATS

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## ABSTRACT

An experiment was planned to study the protective role of aqueous garlic extract (AGE) on tissue anti-oxidant system malondialdehyede (MDA) superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) concentrations in Chromium Chloride ($CrCL_3$) challenged male Wistar rats (n= 162). The animals in nine treatments including three levels of AGE (0, 60 and 120 mg/rat/day) and $CrCL_3$ (0, 4 and 8 mg/kg diet) were fed (over a period of 4 weeks). At the end of feeding trail, six rats per treatment were selected randomly and their blood was collected for analyses. The results showed that different levels of AGE and $CrCL_3$ supplement, significantly increased the content of SOD, GPX and CAT ($P<0.05$) and decreased the concentration of MDA ($P<0.05$). Furthermore, the study of combinative effects of AGE and $CrCL_3$ showed that anti oxidant enzymes levels (SOD, GPX and CAT) and MDA were higher and lower when compared to control groups, respectively ($P<0.05$).This study suggested that addition of AGE and $CrCL_3$ had positive effects on antioxidant defense mechanisms and reduce the susceptibility of blood to free radical oxidative damage.

**Key words**: Garlic, Chromium Chloride, malondialdehyde, tissue anti-oxidants.

## INTRODUCTION

Use of some medicinal plants recently have been common in herbal medicine and have attracted the attention of drug producers. Herbal medicine has been known as scientific one among all nations for many centuries ago, and its therapeutic effects has been proved experimentally. There is no published document about people who consume herbal medicines in the Middle East yet. However, about 15 million people consume herbal medicines and synthetic drugs simultaneously in America (A.S.A, 1999).

Garlic aqueous extract is one of the widely used herbal medicines which is used as an additive in foods. The studies being conducted in herbal medicine area show that consumption of garlic is very useful in treatment of diseases when body defense system becomes weak because of infection. Moreover, Garlic not only prevents against microbes in the respiratory tract, but, it also causes dilation of the mucosa of the lungs. Antioxidant and antigen properties of garlic have been proved recently in a study conducted on livestock and poultry (Bunting *et al*., 2000). Antioxidative and antigenic properties of aqueous garlic have been proved in studies conducted on animal and poultry (Jaffari *et al*., 2006). Most of mentioned properties are related to a combination which is called alliin. Alliin is an odorless chemical from amino acid cysteine family. When garlic aqueous is ground, its alliin is transformed into another material called allicin. Allicin is an active material which gives odor and some therapeutic properties to garlic aqueous extract (Nakagawa *et al*., 1989)

On the other hand, Chromium is one of the heavy metals which effects have not been considered in researches, when is used as a food supplementation. Moreover, it has been shown that chromium rate in diets is in the range of 0.010- 4.2 mg/kg food dry matter. Also, grains containing little amount of chromium and legumes have more amount of this element. Various types of organic chromium (picolinate chromium and nicotinate chromium) and mineral chromium are produced industrially. 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 (Underwood and Suttle,1999). 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 (Underwood and Suttle, 1999; Pechova and Pavlata, 2007). The chromium has important role in the body metabolic and heat stress; so, nowadays it is used in food as supplementation.

The current research was carried out to investigate the combined effect of different levels of garlic extract and chromium chloride supplementation on serum antioxidant parameters such as MDA, SOD, GPX and CAT.

## MATERIALS AND METHODS

**Rats and Management;** This study was carried out as 3×3 factorial experiment with 3 levels of oxidized AGE (0, 60 and 120 mg/rat/day) and 3 levels of $CrCL_3$ supplement (0,4 and 8 mg/ kg diet) in 9 groups, 3 replicates and 6 rats in each replicate in a completely randomized design.

In this experiment, 162 mature male rats (250 gm on the average) were acquired from Razi Serum – producing Institute of Karaj-Iran and transferred to keeping place. All of keeping cages were disinfected before performing the experiment. All of groups were kept in 12-hour light and 12-hour darkness conditions at 25-30$^C$ 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 AGE and $CrCL_3$ supplement:** Local fresh garlic was used in this experiment and garlic extract was added in the soxhlet machine with deionized distilled water for 6 hours on two consecutive days at 30 ° C (to prevent degradation of garlic active components). Then the extract was placed in the incubator for concentrating. Required concentrations of garlic extract were dissolved in distilled water and were administrated daily to the mice (60 and 120 mg/rat/day with 30% concentration) according to the instructions of Jaffari *et al*., 2006. Supplemental chromium chloride (Merck, Germany) was purchased and after the assessment of required amount (4 and 8 mg in kg allotment), were given daily to the rats measured by digital scale. It is important to mention that a garlic extract as gavage (gastro – oral) was feed and the calculated amount of chromium chloride supplement (4 and 8 mg/kg of diet) was dissolved in water after making pellets wet and powder and was added to the food. After stirring and drying food, resulted pellets were given to the animal. Moreover, during the first week of experiment, all groups consumed basal diet in order to adapt with breeding environment conditions; then, fed within 4 weeks on a daily basis (table1).

**Assessment of biochemical factors;** At the end of fourth week, after 12 hours starvation, six rats per treatment were selected randomly from every treatment and their blood was collected for serum concentration of and SOD, GPX, CAT and MDA enzymes. The secondary oxidation product (MDA) was measured according to the tiobarbitoric acid (TBA) method described by Buege and Aust *et al*. (1978).In this method, MDA reacts with TBA to yield a pink-coloured product. The absorbance of 3 ml coloured layer was measured at 335 nm spectrophotometrically. Also, estimation of serum antioxidant enzymes (CAT, GPX and SOD) were done by the method of Aebi (1984); Paglia and Valentine, 1967; Woollliams *et al*., 1983, respectively.

**Statistical analysis:** Data were subjected to a one-way analysis of variance using the General Linear Models (GLM), and the statistical analysis system (SAS) User's guide (2000). 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 GAE levels ( i = 1, 2, 3)

$\beta j$ = The fixed effect of CrCL3 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 AND DISCUSSION

The independent and combinative effects of GAE and $CrCL_3$ supplement, on MDA, SOD, GPX and CAT of serum is presented in Table 2. The obtained results showed that different levels of GAE and $CrCL_3$ supplementation, significantly increased the content of SOD, GPX and CAT ($P<0.05$) and decreased the concentration of MDA ($P<0.05$). Moreover, the study of interaction effects of GAE and $CrCL_3$ showed that anti oxidant enzymes levels (SOD, GPX and CAT) were higher and MDA was lower in compared with control group, respectively ($P<0.05$), which might be due to the Cr element and Garlic that can act as an Anti-oxidant. It is observed that experimental diets have the levels of 60, 120 mg/rat/day GAE and 4, 8 mg/kg $Crcl_3$ had the most effect on the rate of above-mentioned chemical ultra measures compared with the treatment lacked of GAE and $Crcl_3$ supplement. Moreover, in the present research, reduction of MDA concentration of serum (peroxidation index) was remarkable by increase of antioxidant enzymes rate in experimental groups.

Antioxidant enzymes, i.e. superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), operate in concert together with several non-enzymatic molecules to contrast the ROS actions and to avoid oxidative damage. SOD catalyzes the reduction of superoxide anion into hydrogen peroxide, which is subsequently detoxified by CAT and GPx at both intra- and extracellular levels. Three different isoforms of SOD have been identified: SOD1 (Copper Zinc-SOD, CuZn-SOD), localized in cytoplasm, which includes Cu and Zn at its catalytic site, SOD2 (Manganese-SOD, Mn-SOD), localized in mitochondria, which have Mn as a co-factor, and finally SOD3 (EC-SOD), an extracellular antioxidant enzyme, which, such as SOD1, presents Cu and Zn at its catalytic site. Other transition metals are important components of the enzymatic antioxidant defenses: Cu and iron (Fe) are, in fact, essential components of CAT,

and selenium (Se) is a constitutive component of GPx. Consequently, metal ions are crucial for life.

Khatua *et al*. (2012) stated that dose dependent increase in serum NO level was observed after garlic 250 and 500mgkg (-1) dose feeding. While no change in serum SGPT and SGOT level, a significant decrease in serum LDH level was observed after garlic feeding. Garlic-induced NO formation was further confirmed in human aortic endothelial cells (HAEC). Administration of isoproterenol caused a significant decrease in endogenous antioxidants i.e., myocardial catalase, GSH and GPX activity, and mitochondrial enzyme activities like citrate synthase and β hydroxyacyl CoA dehydrogenase. All those deleterious cardiac changes induced by isoproterenol were significantly attenuated by garlic homogenate. However this beneficial effect of garlic was blunted when garlic was administered with L-NAME, a nonspecific inhibitor of nitric oxide synthase (NOS).

**Table1. Experimental groups**

| | |
|---|---|
| **G 1** | **basal diet** |
| G 2 | basal diet + 60 mg/rat/day fresh AGE |
| G 3 | basal diet + 120 mg/rat/day fresh AGE |
| G 4 | basal diet + 4 mg/ kg diet $C_rcl_3$ |
| G 5 | basal diet + 8 mg/ kg diet $C_rcl_3$ |
| G 6 | basal diet + 60 mg/ rat/day fresh AGE + 4 mg/ kg diet $C_rcl_3$ |
| G 7 | basal diet + 60 mg/ rat/day fresh AGE + 8 mg/ kg diet $C_rcl_3$ |
| G 8 | basal diet + 120 mg/ rat/day fresh AGE + 4 mg/ kg diet $C_rcl_3$ |
| G 9 | basal diet +120 mg/ rat/day fresh AGE + 8 mg/ kg diet $C_rcl_3$ |

**Table 2: Biochemical traits of serum from rats fed diets containing supplementary AGE and $CrCl_3$.**

| | | MDA (nm/Ml) | CAT (IU/g Hb) | GPX (IU/g Hb) | SOD (IU/g Hb) |
|---|---|---|---|---|---|
| **Supplementary AGE** | | | | | |
| **0 mg /rat/day (control)** | | 6.94 $^{c}$ | 170.51 $^{c}$ | 98.73 $^{c}$ | 106.35 $^{c}$ |
| **60 mg /rat/day** | | 5.01 $^{b}$ | 191.65 $^{b}$ | 106.93 $^{b}$ | 18.12 $^{b}$ |
| **120 mg /rat/day** | | 3.06 $^{a}$ | 221.31 $^{a}$ | 120.41 $^{a}$ | 2.23 $^{a}$ |
| **P** | | * | * | * | * |
| **SEM** | | 0.05 | 4.23 | 5.65 | 2.12 |
| **Supplementary $CrCl_3$** | | | | | |
| **0 (control)** | | 8.21 $^{c}$ | 55.29 $^{c}$ | 90.17 $^{a}$ | 150.55 $^{a}$ |
| **4 mg /kg diet** | | 7.01 $^{b}$ | 56.19 $^{b}$ | 99.1 $^{b}$ | 173.43 $^{b}$ |
| **8 mg /kg diet** | | 5.50 $^{a}$ | 55.46 $^{a}$ | 123.05 $^{c}$ | 191.01 $^{c}$ |
| **P** | | * | * | * | * |
| **SEM** | | 0.05 | 4.23 | 5.65 | 2.12 |
| **Supplementary AGE** | **Supplementary $CrCl_3$** | | | | |
| **0 mg /rat/day** | **0 (control)** | 6.63 $^{a}$ | 139.01 $^{a}$ | 75.01 $^{a}$ | 110.12 $^{a}$ |
| | **4 mg /kg diet** | 4.91 $^{b}$ | 155.59 $^{b}$ | 121.29 $^{a}$ | 117.04 $^{b}$ |
| | **8 mg /kg diet** | 4.80 $^{b}$ | 178.70 $^{c}$ | 128.10 $^{b}$ | 149.11 $^{c}$ |
| **60 mg** /rat/**day** | **0 (control)** | 5.70 $^{a}$ | 161.19 $^{b}$ | 110.11 $^{a}$ | 115.23 $^{a\,b}$ |
| | **4 mg /kg diet** | 5.01 $^{ab}$ | 160.20 $^{b}$ | 115.15 $^{b}$ | 132.34 $^{cd}$ |
| | **8 mg /kg diet** | 4.91 $^{ab}$ | 159.48 $^{b}$ | 116.61 $^{b}$ | 130.65 $^{cd}$ |
| **120** mg **/rat/day** | **0 (control)** | 4.02 $^{b}$ | 169.19 $^{bc}$ | 125.77 $^{bc}$ | 145.01 $^{c}$ |
| | **4 mg /kg diet** | 3.91 $^{b}$ | 180.19 $^{bc}$ | 152.40 $^{d}$ | 151.08 $^{e}$ |
| | **8 mg /kg diet** | 3.51 $^{e}$ | 192.22 $^{d}$ | 168.04 $^{e}$ | 164.55 $^{e}$ |
| **P** | | * | * | * | * |
| SEM | | 0.98 | 6.52 | 4.81 | 5.06 |

Mean ± Standard deviation, NS = Not significant (P > 0.05), * = P < 0.05. Means with different superscripts within the same column and for the same parameter are significant (P<0.05).

Shaik *et al*. (2008) demonstrated that warm IR injury caused a significant increase in the plasma markers of liver injury, which was attenuated by diallyl sulfide (DAS, a garlic active constituent). The hepatoprotective effects of DAS were associated with significant reductions in lipid peroxidation markers and in situ

generation of superoxide in the liver and increases in the glutathione levels of the liver and bile, suggestive of an antioxidant effect for DAS. Additionally, DAS caused an almost twofold increase in the protein expression of the liver heme oxygenase-1, an enzyme that confers cytoprotection against oxidative stress.

In a research conducted by Kusal (2009), Reviews has shown garlic will lead to reduce toxicity of heavy metals. Garlic has also the ability to stimulate the lymphatic system which expedites the removal of waste from the body. The garlic can be an effective material to protect cells against free radicals as it contains a number of amino acids such as Cysteine, Glutamine, Isoleucine and Methionine that are involved in producing antioxidant enzymes. Also, Nakagawa (1989) reported in another research that most of garlic aqueous effect relates to a compound called allin which is an adourless chemical compound from cysteine amino acid family. Besides, Richard *et al* (2001) have reported that Cr as Cr pidolate (tris chromium III) supplement led to a significant decrease in the plasma MDA of people with type 2 diabetes, which might be due to the effect of Cr element on the glucose/ insulin system. In addition, in other research conducted that Chromium, like vitamin E, has been shown to protect rats from oxidative damage related to carbon tetrachloride exposure (Tezuka,1991), and also decreases lipid peroxidations in isolated rat hepatocytes. Also, Hepatic and renal TBARS were also reduced in hypertensive rats receiving Cr as polynicotinate (Preuss, 1998).

**Conclusion:** The use of $CrCL_3$ and AGE in feed mixes given to rats in a period of 4 weeks increases the anti-oxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and serum catalase (CAT) and decrease MDA content. Moreover, it was better at higher levels of $CrCL_3$ (8 mg/kg diet) and AGE (120 mg/rat/day). Finally, it is concluded that the usage $CrCL_3$ and AGE in the rats' diet may reduce the susceptibility of blood to free radical oxidative damage.

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