

## Anti-hepatotoxic Activity of Garlic (*Allium sativum*) Aqueous Extract compared with Chromium Chloride in Male Rats

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**Abstract:** This study was carried out to show the anti-hepatotoxic activity of garlic in Rats. 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. This design is performed as a factorial experiment 3\*3 (3 level of GAE extract and 3 level of CrCl<sub>3</sub> supplement) in the form of totally random design with 9 groups per 3 replications each containing 6 rat. 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 AST and ALT were determined. Results showed that Garlic improves the cell injury by enhancement of AST and ALT levels and this action increases when CrCl<sub>3</sub> is combined.

**Key words:** Garlic (*Allium sativum*), Chromium Chloride, AST, ALT, Rat.

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### INTRODUCTION

Garlic is probably one of the earliest known medicinal plants (Lewis and Elvin-Lewis, 2003). Its bulbs (cloves) had been used as a cure-all in ancient Egypt and are mentioned in the Ebers Papyrus, one of the earliest treatises on medicinal plants. Garlic contains sulfur containing compounds. Alliin, is converted to the anti-microbial active allicin, when the bulb is cut or bruised. Ajoene, which is a secondary degradation product of alliin, is presumably the most active compound responsible for the anti-thrombotic activity of garlic (Wichtl, 2004). The fresh bulb contains alliin, allicin and volatile oils. When the garlic clove is crushed, the odorless compound alliin is converted to allicin, via the enzyme allinase. Allicin gives garlic its characteristic pungent smell (Williamson, 2003). Also, it contains vitamins and minerals (Gruenwald, 2004) and trace elements (selenium and germanium) (Skidmore-Roth, 2003). Garlic has also been shown to have antioxidant properties, which could have a protective nature against gastrointestinal neoplasias, against blood clots (anti-platelet action) due in part to the compounds alliin and ajoene, which have fibrinolytic activity. Ajoene inhibits thromboxane synthesis through the inhibition of the cyclo-oxygenase and lipoxygenase enzymes (Schulz *et al.*, 2004). Garlic oil significantly decrease the levels of antioxidants ceruloplasmin, albumin and total thiols in the plasma of diabetic rats but SOD activities was decreased in tissue homogenates of liver and kidney (Mamdouh and Abdel-Raheim, 2003).

Aspartate aminotransferase (AST) is a widely distributed enzyme, which is found in many tissues and organs, with high activity in the liver (Zimmerman *et al.*, 1968). Increased AST activity in the serum is a sensitive marker of liver damage (Meyer and Harvey, 1998). There are two main isoenzymes: mitochondrial and cytosolic, which prevails in the total concentration in the blood plasma because it has a longer half-life (Kramer and Hoffman, 1997). Activity of AST in horses is much higher than in other animals (Cornelius *et al.*, 1958). In Hafling horses the activity of serum AST amounted  $146.8 \pm 5.6$  U/L (Weigert *et al.*, 1980), while Kaneko *et al.* (1997) mention values of  $296 \pm 70$  U/L. In addition to species, breed and age, AST activity is influenced by muscle activity (Weigert *et al.*, 1980). Working horses have an approximately 60% higher activity (112 IU/L) than horses which are at rest for several days (70 IU/L) (Weigert *et al.*, 1980).

In primates, dog, cat, rabbit and rat, alanine aminotransferase (ALT) is a specific cytosol liver enzyme, and its increase in the blood plasma is specific for changes in the liver, but ALT activity in pigs, horses, goats, sheep and cattle is not specific for the liver, in order to have a diagnostic significance (Kramer and Hoffman, 1997). ALT activity in the blood plasma is influenced by age and muscle activity (Weigert *et al.*, 1980).

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 main objective of present study was to evaluation of Garlic (*Allium sativum*) Aqueous Extract effects on serum value of AST and ALT compared with Chromium Chloride in Male Rats.

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## MATERIALS AND METHODS

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. This design is performed as a factorial experiment 3\*3 (3 level of GAE extract and 3 level of CrCL<sub>3</sub> 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 CrCL<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub>, basal diet + 8 mg/ kg diet Crcl<sub>3</sub>, basal diet + 60 mg/ rat/day fresh GAE+ 4 mg/ kg diet Crcl<sub>3</sub>, basal diet + 60 mg/ rat/day fresh GAE+ 8 mg/ kg diet Crcl<sub>3</sub>, basal diet + 120 mg/ rat/day fresh GAE+ 4 mg/ kg diet Crcl<sub>3</sub> and 120 mg/ rat/day fresh GAE+ 8 mg/ kg diet Crcl<sub>3</sub>, 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 AST and ALT 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 (SAS, 2000) User's guide. 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

$\mu$  = Overall mean

$\alpha_i$  = The fixed effect of GAE levels (  $i = 1, 2, 3$  )

$\beta_j$  = The fixed effect of CrCL<sub>3</sub> levels (  $j = 1, 2, 3$  )

$e_{ijk}$  = The effect of experimental error

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

### **Results:**

Based on table 1, garlic aqueous extract decreases the AST at the dose of 60 mg /kg and increases the value of ALT also AST at the dose of 120 mg /kg.

CrCl<sub>3</sub> also increases the value of AST and ALT at the dose of 4 mg /kg and decreases these markers at the dose of 8 mg /kg.

Concomitant use of garlic and CrCl<sub>3</sub> yields to decrease in amount of AST when garlic is 60 mg /kg and CrCl<sub>3</sub> is 8 mg /kg. Same results obtained about ALT. Also, concomitant use of garlic and CrCl<sub>3</sub> results in significant decrease in AST and ALT values when garlic is 120 mg /kg and CrCl<sub>3</sub> is 8 mg /kg.

**Table 1:** Comparison of data obtained from analyzing of serum values of AST and ALT

Garlic		AST	ALT
0 mg /kg (control)		149.60	86.80 <sup>b</sup>
60 mg /kg		135.41	88.05 <sup>b</sup>
120 mg /kg		152.61	102.92 <sup>a</sup>
P		0.19	0.007
SEM		7.21	3.94
SupplementaryCrCl <sub>3</sub>			
0 (control)		149.94	92.76
4 mg /kg		154.79	97.35
8 mg /kg		129.57	85.50
P		0.03	0.07
SEM		7.21	3.94
Interaction			
Garlic	Supplementary CrCl <sub>3</sub>		
0 mg /kg	0 (control)	152.33	88.50
	4 mg /kg	157.80	89.40
	8 mg /kg	135.25	91.00
60 mg /kg	0 (control)	131.71	89.42
	4 mg /kg	152.00	89.80
	8 mg /kg	124.00	84.40
120 mg /kg	0 (control)	178.25	105.00
	4 mg /kg	154.50	116.75
	8 mg /kg	130.60	90.20
P		0.44	0.63
SEM		23.90	6.84

### Discussion and Conclusion:

Liver function tests (LFTs or LFs), are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver (American Gastroenterological Association position statement, 2002). The parameters measured include PT/INR, aPTT, albumin, bilirubin (direct and indirect) and others. According to some, liver transaminases (AST/ALT (SGOT/SGPT) are not liver function tests, but are biomarkers of liver injury in a patient with some degree of intact liver function. Other sources include transaminases (AGA Technical review on the evaluation of liver chemistry tests, 2002). Most liver diseases cause only mild symptoms initially, but it is vital that these diseases be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. This testing is performed by a medical technologist on a patient's serum or plasma sample obtained by phlebotomy. Some tests are associated with functionality (e.g., albumin); some with cellular integrity (e.g., transaminase) and some with conditions linked to the biliary tract (gamma-glutamyl transferase and alkaline phosphatase). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to 1) detect the presence of liver disease, 2) distinguish among different types of liver disorders, 3) gauge the extent of known liver damage, and 4) follow the response to treatment. Some or all of these measurements are also carried out (usually about twice a year for routine cases) on those individuals taking certain medications — anticonvulsants are a notable example-in order to ensure that the medications are not damaging the person's liver.

Sajitha *et al.*, 2010 stated that daily feeding of drinking water containing lead acetate (160 mg/l) or 10% alcohol by volume or a combination of both to rats for a month produced certain deleterious effects through oxidative stress. They showed that both heavy metal lead and alcohol are capable of doing such damages. The deleterious alterations observed were in the parameters of blood, serum and tissues, viz; Hb, Pb, proteins, lipids, lipid per oxidation, Vitamins C and E levels and enzyme activities of AST, ALT, and catalase. Simultaneous feeding of either of the two antioxidants garlic oil (GO) and vitamin E at equal doses of 100 mg/kg/day, to the rats counteracted the deleterious effects of the above two chemicals significantly. The maximum damage was brought about by feeding of drinking water containing both lead acetate and alcohol. The protective effects of GO and Vitamin E were not significantly different. They concluded that mechanism of actions of the Vitamin E and GO is probably due to their efficiency as detoxifying agents and antioxidants, to scavenging free radicals as well as an independent action of GO on the removal of lead salt as lead sulfide, that is compatible with our research results.

Zhang *et al.*, 2010 also showed that compared with negative group, the liver coefficient and the activities of ALT and AST in serum of model group were increased remarkably ( $P < 0.01$ ). Compared with CCl<sub>4</sub> model group, the liver coefficient and the activities of ALT and AST in serum were decreased significantly ( $P < 0.01$ ) by garlic oil dose-dependently in each preventive group. Simultaneously, histological assessment showed that garlic oil effectively alleviated hepatocyte injuries induced by CCl<sub>4</sub>. Comparing the preventive effects of garlic oil in every group, it was better in preventive group 3 than others. However, all indexes and histological examinations in therapeutic group 1 did not show the difference with those of CCl<sub>4</sub> model group. In therapeutic group 2, all indexes recovered after 5 d of CCl<sub>4</sub> administration.

Obioha *et al.*, 2009 observed a decrease in hepatic activities of alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase and a concomitant increase in the plasma activities of ALT and AST. Onion and garlic extracts significantly attenuated these adverse effects of Cd. Onion extract proffered a dose-dependent hepatoprotection. Our study showed that Cd-induced oxidative damage in rat liver is amenable to attenuation by high dose of onion and moderate dose of garlic extracts possibly via reduced lipid peroxidation and enhanced antioxidant defense system that is insufficient to prevent and protect Cd-induced hepatotoxicity.

Sener *et al.*, 2005 were determined serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels to assess liver functions. They were taken liver tissues for the determination of malondialdehyde (MDA) levels, an end product of lipid peroxidation; glutathione (GSH) levels, a key antioxidant; and myeloperoxidase (MPO) activity, as an indirect index of neutrophil infiltration. Hepatic collagen content, as a fibrosis marker, was also determined. Plasma ALT and AST activities were elevated in the I/R group as compared with the control group, while these increases were significantly decreased by AGE treatment. Hepatic GSH levels, significantly depressed by I/R, were elevated back to control levels in the AGE-treated I/R group. Increases in tissue MDA levels and MPO activity due to I/R injury were reduced back to control levels by AGE treatment. Similarly, increased hepatic collagen content in the I/R group was reduced to the control level with AGE treatment. Since AGE administration alleviated the I/R-induced injury of the liver and improved the hepatic structure and function, it seems likely that AGE, with its antioxidant and oxidant-scavenging properties, may be of potential therapeutic value in protecting the liver against oxidative injury due to ischaemia-reperfusion. It can be concluded that Garlic improves the hepatic cell injuries by enhancement the AST and ALT activity and this action increases when CrCl<sub>3</sub> is combined.

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