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The antioxidant effect of garlic powder on rats treated by different doses of chromium chloride

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Abstract

The present study was done to illustrate the antioxidant effect of garlic powder on rats' received different doses of $CRCl_3$. Male rats' n=60 were divided to 6 groups each of 10 rats. The 1^{st} group kept as control. The other groups received $Crcl_3$ dissolved in drinking H_2O (1mg/l and 10mg/l) and garlic powder 5% mixed with diet as follows. 2^{nd} group ($Crcl_3$ 1mg/l), 3^{rd} group ($Crcl_3$1mg/l + 5% garlic powder), 4^{th} group ($Crcl_3$ 10 mg/l), 5^{th} group ($Crcl_3$10 mg/l + 5% garlic powder) and 6^{th} group (5% garlic powder). After 45 days rats were scarified for analysis of serum and tissue (liver and kidneys) antioxidant enzymes (GPx, SOD and MDA) and minerals (Cu, Zn and Fe). Results showed marked elevation of GPx and SOD in groups received $Crcl_3$ and garlic powder with decrease in MDA level in serum and tissue. Cu and Zn levels were increased in serum and liver tissue of most treated groups while Fe level was marked decreased in serum and tissue of most treated group. From our results we conclude that $Crcl_3$ and garlic has cooperative effect enhancing the antioxidant system and decreasing lipid peroxidation of rats. This may play an important role on improving the body health, competing free radicals that may result from exposure to envirmental pollutants which harm human and animals health.

Introduction

Chromium is a naturally occurring heavy metal found commonly in the environment in trivalent and hexavalent forms.**(Shrivastava *et al*., 2002**).

Commercially chromium compounds are found in organic and inorganic salt (**World Health Organization**, **2009**). Trivalent chromium is safe and has antioxidant activity (**Preuss *et al*., 2008 and Chen *et al*., 2010**).

Chromium chloride has been known to be a micronutrient for mammals, the highest concentration was found in most products of oils, fats, cereals, carats, potatoes and spinach (**Eisenterg *et al*., 1998 and O´connell,2001**).

$CrCl_3$ decreases oxidative stress and lipid peroxidation (**Jain and kannan,2001**). It was famed that higher doses of chromium induce more patent anabolic and antioxidant effect (**Siripurkpang and Bangchauj, 2009).**

Garlic (Allium sativum) is one of the herbal medicine which is used as additive in foods. Garlic is one of the members of family (genus Allium) which includes garlic, scolious, onions and leeks. These ingredients contain the sulfa compounds which are medicinally active (**Ghalehkandi *et al*., 2013**).

Many studies show that consumption of garlic is very useful in treatment of diseases when body defense system become weak. Antioxidant and antigen properties of garlic have been proved recently in a study achieved in animal and poultry (**Jaffari *et al*., 2006**).

Most of garlics properties are related to allin. Allun is an odorless chemical amino acid (cysteine) form. Formality when garlic ground, its allin is transferred to allicin. Allicin is an active material which gives odor and therapeutic properties to garlic (**Nakagawa *et al*., 1989**).

The current study was carried out to investigate the combined action of different levels of chromium and garlic extract on serum and tissue antioxidant agents' malaneliadeyle (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) as well as its effect on some essential elements as copper, zinc and iron.

Materials and methods

A-Dosing:

Chromium chloride ($CrCl_3$):

It made in Ameco –B - USA with chemical formula $CrCl_3$ and molecular weight 122.90, dissolved in water.

Garlic powder: was purchased from Ameca. Bias company, USA.

Garlic powder: was given according to **Sanghui. K(2003)**

Chromium chloride was given according to **Ghalehkand. et al (2013)**

B-Experimental Design:

A total number of 60 male albino rats weighting 120-150 gm body weight were purchasing from laboratory animals breeding unit, national research Center, Dokki, Egypt.

The rats were housed under hygienic conditions received normal diet and water was given ad libitum. Rats were divided into 6 groups, 10 rats for each. 1st group kept as control (-ve control), 2nd group was given chromium chloride $CrCl_3$ at dose of 1mg/lite of drinking water,3rd group was given ChCl3+ garlic powder 5% mixed with diet, 4th group was given $CrCl_3$ at dose of 10mg/lite of drinking water, 5th group was given $CrCl_3$ at dose of 10mg/lite + garlic powder 5% mixed with diet and 6th group given garlic powder 5% mixed with diet as +ve control).

C- Samples preparation:

At the end of the experiment, rats were anesthetized and scarified and blood samples were collected and incubated until the blood clotted then the samples were centrifuged at 3000 rpm for 15 min. and the clear serum was separated and stored at -20ºC till biochemical analysis.

Internal organs (liver and kidney) were collected and stored in -20ºC till biochemical analysis.

D- Methods:

GPx activity was determined according to the method designed by **Aebi (1984**), the assay of super oxidase dismutase (SOD) activity was carried according to **Bannister and Calebrese(1987).**

Lipid peroxide was determined by lipid peroxidase assay kit Randax, England as Malondialdehyde (MDA) according to **Satoh (1978).**

E- Estimation of copper, zinc and iron:

1- Preparation of samples:
- Tissue samples: digestion procedure was carried out according to (**Mirranda *et al*.,2005**).
- Serum samples were diluted by using 6% butanol.

2- Determination: instrument procedure for analysis various heavy metals were based on those suggested in the manual of the flame Atomic Abssorpation spectrophotometer (UNICAM969AA Spectronic,USA).

Contents of tissue (liver and kidney) according to **Miranda *et al*. (2005).**

F- Statistical analysis:

Data collected were subjected to analysis of variana (ANOVA). All statistical calculations were performed with IBM Spss statistics program version 20.

Results and Discussion

The independent and combination effects of CrCl3 and garlic supplement on GPx, SOD and MDA in serum of rats is illustrated in Table (1). The obtained results showed an elevation in the level of GPx and SOD in groups received Cr_1, Cr_1+garlic and Cr_2+garlic, meanwhile the level of MDA in the same groups decreased.

Analysis of liver tissue (Table 2) revealed that there is an increase in the level of GPX and SOD enzymes with decrease in MDA enzyme in all groups compared with control. Meanwhile, analysis of kidney tissue (Table 3) showed an elevated level of GPX and SOD enzymes with decrease in MDA enzyme in groups received high dose of chromium chloride with garlic where there is a decrease in the level of SOD in group received low $CrCl_3$ dose.

Our results come in agreement with these reported by **Chalehkndi and Ebrahimnezhad *et al*., (2013)** and **Ibrahim *et al*.,(2011)** but are different from those found by **Vliza *et al*., (2014).**

SOD (superoxidase dismutase) is one of the enzymes that interrupted the chain of oxygen-dependent free radical reactions in case of aerobic organisms, so enzymes activity is associated with intensity of lipid peroxidation (**Poberezkina and Osiuskaia,1989**), there was an increase in the activity of GPx which participated with reduction of H_2O_2, a product of the reaction of SOD with active forms of oxygen (free radicals) (**Galecka *et al*.,2008).**

Catalase and peroxidase as GPx remove hydrogen peroxide molecules which are by- product of the reaction produced by SOD from tissues and convert it to water, preventing both cell damage and the formation of other more toxic free radicals. In this way superoxide free radical and hydrogen peroxide are converted to the harmles product and water.

Lipid peroxides (LP) as MDA occurred usually with toxic matters in the body, LP react with molecules as membrane proteins in the body leading to damage of cell membranes. Cr and garlic lead to decrease the level of MDA as showing in this study. This is accordance with those reported by (**Chalekandi *et al*.,2013)**.

Garlic (Allium sativum) is a member of Liliaceae family, it has antiviral, antifungal, anticancer and antioxidant capacity. The genus allium contains the sulfur compounds which are medicinally active. The most abundaut sulfur compound in garlic is alliun. S-alkyl cysteine-sulfoscides) which present at 10mg/g in fresh garlic or 30mg/g in dry matter (**Lawson, 1998**). Garlic can be an effective materials to protect cells against free radicals as it contains number of amino acids such as systein, glutamine and methionine that are involved in producing antioxidant enzymes. Feeding the cysteine diet causes higher activity of total and Zn-superoscide dismutase and catalase of liver (**He and Aoyama, 2003**).

Garlic enhances serum level of catalase and glutathione peroxidase (**Prasad *et al*.,1995).** Garlic extract allicin is efficiently escovengedd escogenauslly generated hydroxyl radicals in a dose dependent fashion, also other garlic as constituents S-alkylcysteine produced significant antioxidant effects (**Tarok *et al*., 1994).**

Regarding the effect of Cr and garlic on level of Cu, Zn and Fe in serum of rats (Table 4) showed a marked increase in Cu and Fe levels ingroups of rats treated with low $CrCl_3$ dose.

Mineral concentration (Cu, Zn and Fe) in liver tissue of rats was illustrated in Table (5) that showed marked increase in the level of copper of most groups while zinc level was increased only in group that received low Cr dose. Meanwhile, Fe level was declined in most groups compared to control.

Chromium is considered to be relatively nontoxic and it is widely accepted an essential microelement in animals and human nutrition (**Zhitkovich, 2011**) and **Lee *et al*.(2012).** Chromium supplement in basal diets effects the other elements, with **a** synergistic effect on copper level in serum **(Sahin *et al*.,2007).** Chromium supplementation causes an increase in Cu and Zn levels that activates liver enzymes synthesis of Cu/Zn-SOD (**Pechova *et al*.,2002**)

This is probably contributes to that an increase in the activity of SOD and sequencing of superoxide radical, indirect produce an effect on the activity of catalase and GPx (**Ramachandion *et al*.,2004)**. **Chen *et al*.(2010)** confirmed that Cr play a role in Cu/Zn-SOD, CAT and GSH-PX gene expression.

The lowering effect of chromium on level of Fe may be due to that Cr competes on B-site of transferrin (**Stearns, 2000**).

Hexavalent chromium induces hematological signs of microcytic anemia in rodents. Considering that chromium can oxidize ferrous (Fe^{2+}) to Ferric (Fe^{3+}) iron in the lumen of small intestine and perturbs iron absorption exposure to Cr (VI) in drinking water resulted in dose – dependent decrease in Fe levels in the duodenum , lever serum and bone (Food and chemical toxicology, 2014).

Concerning the role of garlic on mineral levels garlic contains many active ingredients as organic-sulfur compound as allin and allicin, their effects on the mineral bioavailability have been tested because of their antioxidant effect that protect some important mineral against the oxidative damage and prevent the oxidation of some important fatty acids that enhance the mineral bioavailability (**Chatty *et al*., 2004)**.

Our results investigate the important role of $CrCl_3$ and garlic in elevation and improvement of body health. These by decreasing the effect of free radicals and lipid peroxidation resulted from the exposure to environmental pollutants and stressed factors that harm human and animals.

Table (1): effect of chromium chloride and garlic as antioxidant activity and lipid peroxides in rat serum

parameters	Group1	Group2	Group3	Group4	Group5	Group6
GPX(u/ml)	15.33± 0.326 ^{a}	16.11± 0.349 ^{b}	17.59± 0.509 ^{a}	15.85± 0.469 ^{a}	17.83± 0.292 ^{b}	15.08± 0.31 ^{a}
SOD(u/ml)	17.11± 0.383 ^{a}	18.32± 0.30 ^{b}	19.31± 0.124 ^{bc}	17.74± 0.47 ^{bc}	21.69± 0.51 ^{d}	16.74± 0.3 ^{b}
MDA(nmal/ml)	3.81± 0.07 ^{a}	2.78± 0.217 ^{b}	2.84± 0.085 ^{bc}	2.39± 0.187 ^{cd}	2.28± 0.129 ^{d}	2.07± 0.163 ^{a}

Data are represented as means of 10 samples, a,b,c: significant differences between groups (rows) ($p<0.05$).Each result is expressed as mean + SE and corresponds to the analysis performed by Duncan Multiple range test.

Table (2): Effect of chromium chloride and garlic as antioxidant activity and lipid peroxides in rat liver

parameters	Group1	Group2	Group3	Group4	Group5	Group6
GPX(u/gm)	20.80± 0.904[a]	25.89± 0.224[b]	23/91± 0.259[c]	30.63± 0.713[d]	33.08± 0.610[e]	55.44± 0.343[f]
SOD(u/gm)	19.21± 0.04[a]	19.76± 0.249[a]	20.2± 0.223[b]	22.09± 0.407[c]	26.45± 0.043[d]	24.46± 0.93[b]
MDA(nU/gm)	5.65± 0.24[a]	4.5± 0.256[b]	4.02± 0.302[bc]	4.7± 0.199[cd]	4.19± 0.132[d]	4.09± 0.118[d]

Data are represented as means of 10 samples, a,b,c: significant differences between groups (rows) ($p<0.05$).Each result is expressed as mean + SE and corresponds to the analysis performed by Duncan Multiple range test.

Table (3): Effect of chromium chloride and garlic as antioxidant activity and lipid peroxides in rat kidney

parameters	Group1	Group2	Group3	Group4	Group5	Group6
GPX(u/gm)	3.5± 0.078[a]	3.75± 0.14[a]	3.78± 0.092[a]	3.68± 0.124[a]	4.35± 0.137[b]	5.24± 0.228[c]
SOD(u/gm)	11.75± 0.183[a]	11.36± 0.228[a]	9.91± 0.174[b]	12.29± 0.361[a]	13.72± 0.431[c]	10.65± 0.141[b]
MDA(nU/gm)	3.34± 0.17[a]	4.19± 0.112[b]	3.36± 0.124[a]	3.04± 0.06[a]	2.75± 0.140[c]	2.81± 0.072[c]

Data are represented as means of 10 samples, a,b,c: significant differences between groups (rows) ($p<0.05$).Each result is expressed as mean + SE and corresponds to the analysis performed by Duncan Multiple range test.

Table (4): Effect of chromium chloride and garlic on copper (cu), zinc (zn) and Iron(Fe) in rats serum

parameters	Group1	Group2	Group3	Group4	Group5	Group6
Cu(ppm)	0.227± 0.0122[a]	1.36± 0.071 [b]	0.152± 0.001 [b]	0.126± 0.003 [a]	0.257± 0.009 [b]	0.45± 0.006 [c]
Zn (ppm)	0.482± 0.141 [a]	0.505± 0.0188 [b]	0.393± 0.047[bc]	0.692± 0.232 [c]	0.349± 0.131 [b]	0.633± 0.217 [b]
Fe (ppm)	0.699± 0.028 [a]	0.287± 0.0189 [b]	1.75± 0.381 [bc]	0.585± 0.214 [cd]	0.114± 0.010 [d]	0.163± 0.202 [d]

Data are represented as means of 10 samples, a,b,c: significant differences between groups (rows) ($p<0.05$).Each result is expressed as mean + SE and corresponds to the analysis performed by Duncan Multiple range test.

Table (5): Effect of chromium chloride and garlic on copper (cu), zinc (zn) and Iron(Fe) in rats liver

parameters	Group1	Group2	Group3	Group4	Group5	Group6
Cu(ppm)	0.305± 0.005[a]	0.413± 0.008 [b]	0.426± 0.00[9 b]	0.259± 0.003 [c]	0.423± 0.019 [b]	0.355± 0.020 [a]
Zn (ppm)	0.446± 0.052 [a]	1.223± 0.031 [b]	0.665± 0.012[a]	0.596± 0.044 [a]	0.715± 0.073 [a]	0.491± 0.019 [a]
Fe (ppm)	6.001 ± 0.265 [a]	5.14± 0.0871 [a]	4.46± 0.143 [b]	2.465± 0.099 [c]	1.496± 0.132 [c]	4.410± 0.119 [d]

Data are represented as means of 10 samples, a,b,c: significant differences between groups (rows) ($p<0.05$).Each result is expressed as mean + SE and corresponds to the analysis performed by Duncan Multiple range test.

Table (6): Effect of chromium chloride and garlic on copper (cu), zinc (zn) and Iron (Fe) in rats kidney

parameters	Group1	Group2	Group3	Group4	Group5	Group6
Cu(ppm)	0.518± 0.014[a]	0.345± 0.015 [a]	3.147± 0.287 [b]	0.363± 0.011 [a]	0.586± 0.014 [a]	0.486± 0.021 [a]
Zn (ppm)	0.279± 0.052 [a]	0.96± 0.175 [b]	0.315± 0.199[b]	1.341± 0.075 [c]	0.298± 0.027 [a]	0.459± 0.014 [a]
Fe (ppm)	1.379 ± 0.024 [a]	1.99± 0.285 [a]	5.399± 0.262 [b]	1.34± 0.028 [a]	1.830± 0.811 [a]	1.854± 0.0147 [a]

Data are represented as means of 10 samples, a,b,c: significant differences between groups (rows) ($p<0.05$).Each result is expressed as mean + SE and corresponds to the analysis performed by Duncan Multiple range test.

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