

## Effects Of Garlic And Acrylamide On Some Antioxidant Enzymes

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### ABSTRACT

Chips and others fast foods that cooked at high temperatures caused many adverse effects to human health. This study was carried out to investigate the effects of acrylamide (a known mutagenic, neurotoxic and carcinogenic substance) alone or garlic alone or in their combination on lipid peroxidation and some antioxidant enzymes in tissues of rabbits. The rabbits were divided into four groups of ten and fed on different rations in one or in combination for a month. After the feeding period, the animals were sacrificed and levels of glutathione, malondialdehyde and activities of glutathione S-transferase and glutathione peroxidase were measured spectrophotometrically. Acrylamide supplementation caused reduction of both glutathione S-transferase and glutathione peroxidase activities as well as reduced glutathione, while it significantly augmented the lipid peroxidation in brain, liver and kidneys of rabbits. Furthermore, the garlic alone or in combination with acrylamide reversed the above adverse effects. Decreased activity of glutathione peroxidase on garlic supplementation may be due very low garlic concentration that was used in the study. We concluded that, garlic success to averts the oxidative stress induced by acrylamide reflected in decreased lipid peroxidation and increased antioxidant enzymes.

**Key words:** Acrylamide, garlic, lipid peroxidation, antioxidant enzymes, rabbits

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### Introduction

Acrylamide (ACR) is a water soluble vinyl monomer used extensively in the production of polyacrylamide with several uses (Gold and Schaumburg, 2000). It is used in water purification, making of cosmetics, glues and paper, as a soil stabilizer and for the production of polyacrylamide gel electrophoresis (Tareke *et al.*, 2002). It is a contaminant in certain potatoes and grain-based foods cooked at high temperature. Its presence in these foods occurs through maillard reaction between amino acids especially asparagine and certain reducing sugar either glucose and/ or fructose (Stadler *et al.*, 2002). In rodent models, ACR had significant carcinogenic effect and damage to the nervous system (LoPachin *et al.*, 2003). The acrylamide toxicity is considered to be hepatotoxic, genotoxic and causes lipid peroxidation (Abou Donia *et al.* 1993; Mukul Das *et al.*, 1982; Cihak and Vontorkova, 1998).

There is an increased demand for using medicinal plants in therapy "back to nature" instead of using synthetic drugs which have many adverse effects that may be more dangerous than the disease itself (Dubick, 1986). Garlic (*Allium sativum*) is one of the most herbal medicine used since ancient time by many cultures as a medical folk remedy for a variety of illnesses. It is used as antibacterial, antithrombotic, antineoplastic, antipyretic, antifungal, larvacidal, antiviral, antispasmodic, diuretic and against chest pains and intestinal disorders (Dubick, 1986; Lohani *et al.*, 2003). It was speculated that administration of garlic significantly alters cancer development by influencing hepatic biotransformation of enzymes responsible for lipid peroxidation (Guyonnet *et al.*, 2000). Therefore, it was the aim of this study, to assess the adverse effects of ACR on some antioxidant enzymes and the possible use of garlic as a natural protection remedy.

### Materials And Methods

Acrylamide was obtained from Sigma Chemical Co. It was given in drinking water at a dose rate of 0.05%. Garlic was purchased from commercial sources; the outer husks were peeled off before slicing the cloves by a knife. The sliced garlic was dried in hot air oven at 60 °C and moisture content of fresh garlic was determined to be around 72%. The dried garlic was ground and kept in dry glass package until use. Garlic was given in diet at a dose rate of 1%. Commercial rabbit food was obtained from the rabbit centre for breeding, Cairo University, Egypt.

Forty (40) male rabbits of three months old and initial weight 1kg-1.5kg were used to study the effects of dietary inclusion of acrylamide and garlic on some antioxidant enzymes. Efforts were made to treat the rabbits humanely and the protocol used was approved by veterinary authorities in the Ministry of Agriculture and Veterinary services, Cairo, Egypt. All rabbits were housed in metal cages and received diet for two weeks before the start of the experiment for acclimatization and to ensure the normal growth and behavior. The

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experiment was a Completely Randomized Design in which animals in four groups were fed for one month on diets that were formulated. They were given basal diet and water *adlibitum*. The animals were on treatment for one month with daily diets of one or a combination of two or three of the following: basal diet, garlic and acrylamide as shown below. All animals remained healthy throughout the experimental period (one month).

Group I: 10 rabbits were fed basal diet and served as control.

Group II: 10 rabbits were kept on basal diet and water containing 0.05% of acrylamide

Group III: 10 rabbits were given basal diet containing 1% of garlic powder per kg diet

Group IV: 10 rabbits were fed on basal diet containing 1% of garlic powder per kg diet and water containing 0.05% acrylamide. At the end of the experiment and night fasting, rabbits were sacrificed using ice-cold 0.05 M tris- HCl buffer, pH 7.4 containing 0.25 M sucrose and the body organs (brain, liver and kidneys) removed and placed in the same ice-cold buffer. The organs were blotted, dried, weighted and homogenized in the ice-cold buffer with twelve strokes in a tight-fitting potter Elvehagen homogenizer.

#### *Determinations:*

##### *Lipid peroxidation:*

Lipid peroxides such as malondialdehyde (MDA), a product of lipid peroxidation was measured spectrophotometrically after the reaction with thiobarbituric acid (Placer *et al.*, 1966).

##### *Enzyme activities:*

Glutathione S-transferase activity (GST) was determined spectrophotometrically at room temperature at a rate of glutathione conjugation of 1-chloro-2, 4-dinitrobenzene. Glutathione peroxidase (GPx) was determined chemically using cumene hydroperoxide as substrate. Reduced glutathione (GSH) was assayed spectrophotometrically basing on the reductive cleavage of 5,5'-dithiobis 2-nitrobenzoic acid by sulphhydryl group to yield yellow colour with maximum absorbance at 412 nm. The obtained data was statistically analyzed using SAS statistical package and results given as Mean±Standard Deviation (SAS, 1987).

#### *Results:*

The results given in Table1 revealed that the administration of acrylamide significantly increased lipid peroxidation as compared to the control. Increased lipid peroxidation due to acrylamide is expressed by an increase in MDA level in the tissues. Supplementation of basal diet with garlic alone or a combination of garlic and acrylamide reduced lipid peroxidation as shown by a decrease in MDA levels as compared to the control and basal diet supplemented with acrylamide alone.

Table 2 demonstrates that the administration of acrylamide or garlic alone or in combination significantly reduced the activity of glutathione peroxidase (GPx) in the tissues as compared to the control group.

As shown in Table 3, the administration of acrylamide alone in the diet resulted in significant decrease in the activity of GST; whereas garlic alone or in combination with acrylamide caused significant increase in the activity of GST in the tissues.

Table 4 shows that the administration of acrylamide alone resulted in significant decrease in the level of glutathione; whereas garlic alone on in combination with acrylamide resulted in significant increase in the content of glutathione in tissues.

#### *Discussion:*

The result presented in Table1 revealed that the administration of acrylamide in drinking water significantly increased lipid peroxidation as expressed by an increase in MDA levels in tissues. Acrylamide is able to increase lipid peroxidation by inducing oxidative stress with generation of free radicals (Jiazhong *et al.*, 1998). These results are in agreement with other reports that showed an increase in lipid peroxidation in brain and liver upon administration of acrylamide (Srivastava *et al.*, 1983). A recent study on human erythrocytes also shows acrylamide induced MDA formation and a decrease in glutathione peroxidase activity in the erythrocyte (Catalgol *et al.*, 2009).

However, in this study, addition of garlic counteracted the effects of acrylamide. These results come in accordance with others (Banerjee *et al.*, 2001; Takada *et al.*, 1994) who reported significant reduction in TBAS in the liver and kidney upon garlic administration in rats. Also the results agree with those obtained by (Zaghloul, 2001). Garlic decreases lipid peroxidation by scavenging free radicals as well as preventing depletion of glutathione through stimulation of synthesis of cellular antioxidants (Nursal *et al.*, 2005; Banerjee *et al.*,

2002). Therefore, the results show that, the protective effect of garlic extract involves the maintenance of antioxidant capacity in protecting the tissues against oxidative stress.

The enzymatic antioxidant defense systems are the natural protectors against lipid peroxidation. Glutathione peroxidase (GPx) is an important scavenger of H<sub>2</sub>O<sub>2</sub> and prevents the generation of hydroxyl radical and protect the cellular constituents from oxidative damage (Scott *et al.*, 1991). Table 2 demonstrates significant reduction in the activity of glutathione peroxidase in the tissues by acrylamide. This suggests an increased utilization of this antioxidant enzyme with subsequent depletion to counter the increased level of free radicals induced by acrylamide in these tissues. Supplementation of the basal diet with garlic or in combination with acrylamide further reduced the activity of this enzyme in all tissues. This was in agreement with previous studies which reported that garlic at low doses produced a very low level of oxidative stress in liver and kidney (Banerjee *et al.*, 2002). On the contrary, the present finding disagree with those obtained by others who reported, that the administration of diallyl sulfide, one of greatest sulfur compound of garlic, prevented reduction of GPx activity induced by gentamycin in kidneys of rats (Jose *et al.*, 2003). Likewise, administration of diallyl trisulfide increased the activity of GPx in the liver of rats exposed to acute liver injuries by carbon tetrachloride (Fukao *et al.*, 2004).

As shown in Table 3, administration of acrylamide caused significant decrease in glutathione S-transferase (GST) activity in all tissues. These results concur with previous studies which reported a decrease in the activity of GST in rat brain on subsequent exposure to acrylamide (EL-Ballal and EL-Manankhly, 1998). In the contrast, Awad *et al.*, (1998) reported an increased activity of GST after incubation of acrylamide with liver slices. In this study, supplementation of garlic alone or in combination with acrylamide resulted in significant increase in the activity of GST in all tissues. These results concur with those obtained previously who reported that, prophylactic treatment of animals with garlic oil before the administration of iron nitrotriacetate caused a recovery of glutathione depletion with increased activities of antioxidant enzymes (Iqbal and Athar, 1998). Garlic induces synthesis of phase II enzymes and this is dependent on the number of sulfur present as proved by diallyl trisulfide (Fukao *et al.*, (2004). The present findings also agree with those obtained by (Sener *et al.*, 2005).

Table 4 shows that the administration of acrylamide resulted in significant decrease in glutathione levels in all tissues. This is concurs with other reports that showed significant decrease in the level of glutathione (GSH) in brain and liver of rats upon acrylamide administration (Srivastava *et al.*, 1983; Fukao *et al.*, 2004). Acrylamide are electrophilic compounds, which property facilitates them to react with vital cellular nucleophiles possessing SH, NH<sub>2</sub> and OH groups. GSH is a cellular non-protein sulfhydryl molecule, which on administration of acrylamide is accompanied by its significant depletion in cells by reacting with SH group of glutathione. This results in formation of glutathione S-conjugates which is the initial step in the biotransformation of electrophiles (like acrylamide) into mercapturic acid with subsequent excretion in the urine. Hence, the body uses glutathione for detoxification and excretion of acrylamide in the body (Edward, 1975). This study concurs with other reports which reported significant decrease in GSH content and GST activity in corpus striatum of rat brain and liver (Shukla-Pradeep *et al.*, 2002). Supplementation of garlic alone or in combination with acrylamide resulted in significant increase in the content of glutathione in all tissues. These results are in agreement with those obtained previously when garlic was supplemented at a dose rate of 2.0% of diet and resulted in significant increase in total GSH content in liver of rats exposed to diethyl nitrosamine (Sanghur *et al.*, 2003). Garlic increases glutathione levels by contributing numerous sulfur compounds and glutathione precursors (Takada *et al.*, 1994): In conclusion, acrylamide caused many adverse effects in the tissues as is reflected in significant increase in lipid peroxidation, decrease in glutathione levels and decreased activities of glutathione peroxidase and glutathione S-transferase. The administration of garlic alone or in combination with acrylamide significantly lowered lipid peroxidation, and enhanced glutathione levels and activity of glutathione S-transferase but decreased glutathione peroxidase activity probably due to low concentration of garlic which is implicated to only induce low levels of oxidative stress.

**Table 1:** Effect of acrylamide and garlic on lipid peroxidation in the brain, liver and kidneys

Groups	LP, nmol MDA/g Wet tissue		
	Brain	Liver	Kidney
Group I (Control)	106.06 ± 1.71 <sup>cb</sup>	116.1 ± 1.8 <sup>ca</sup>	115.93 ± 1.75 <sup>ca</sup>
Group II (ACR)	186.8 ± 1.32 <sup>aa</sup>	178.94 ± 1.39 <sup>ab</sup>	186.66 ± 1.35 <sup>aa</sup>
Group III (Garlic)	90.66 ± 1.71 <sup>da</sup>	105.3 ± 1.8 <sup>da</sup>	101.7 ± 1.75 <sup>da</sup>
Group IV (ACR + Garlic)	142.96 ± 1.32 <sup>bb</sup>	125.7 ± 1.39 <sup>bc</sup>	148.92 ± 1.35 <sup>ba</sup>

\*Means within the same column and row carrying different letters are significantly different at (P<0.05), ACR= Acrylamide, LP= Lipid peroxidation, MDA= Malondialdehyde.

**Table 2:** Effect of acrylamide and garlic on glutathione peroxidase activity in the brain, liver and kidneys

Groups	GPx IU/g Wet tissue		
	Brain	Liver	Kidney
Group I (Control)	21.64 ± 0.35 <sup>ab</sup>	28.48 ± 0.59 <sup>aa</sup>	23.03 ± 0.41 <sup>ab</sup>
Group II (ACR)	16.25 ± 0.27 <sup>bb</sup>	15.16 ± 0.45 <sup>bb</sup>	20.75 ± 0.32 <sup>ba</sup>
Group III (Garlic)	10.73 ± 0.35 <sup>ca</sup>	11.03 ± 0.59 <sup>ca</sup>	9.67 ± 0.41 <sup>ca</sup>
Group IV (ACR+ Garlic)	8.79 ± 0.27 <sup>da</sup>	8.01 ± 0.45 <sup>da</sup>	7.98 ± 0.32 <sup>da</sup>

\*Means within the same column and row carrying different letters are significantly different at (P<0.05). ACR= Acrylamide, IU= International units, GPx= Glutathione peroxidase.

**Table 3:** Effect of acrylamide and garlic on glutathione S-transferase activity in the brain, liver and kidneys

Groups	GST mol CDNB/min/g Wet tissue		
	Brain	Liver	Kidney
Group I (Control)	3160 ± 118.2 <sup>cb</sup>	4480 ± 0.59 <sup>bb</sup>	7351 ± 51.09 <sup>ba</sup>
Group II (ACR)	1597.2 ± 91.62 <sup>db</sup>	1609.8 ± 0.45 <sup>db</sup>	2039 ± 39.57 <sup>da</sup>
Group III (Garlic)	4942.6 ± 118.2 <sup>ac</sup>	6673.6 ± 0.59 <sup>ab</sup>	8297.6 ± 51.09 <sup>aa</sup>
Group IV (ACR+ Garlic)	3867 ± 91.62 <sup>bb</sup>	3245.4 ± 0.45 <sup>cb</sup>	5269.4 ± 39.57 <sup>ca</sup>

\*Means within the same column and row carrying different letters are significantly different at (P<0.05), GST=Glutathione S-transferase, CDNB= 5.5. dithiobis 2-nitrobenzoic acid, ACR= Acrylamide.

**Table 4:** Effect of acrylamide and garlic on glutathione level of brain, liver and kidneys

Groups	GSH μmol/g Wet tissue		
	Brain	Liver	Kidney
Group I (Control)	25.49 ± 0.60 <sup>bb</sup>	63.02 ± 1.73 <sup>ca</sup>	51.95 ± 2.47 <sup>ba</sup>
Group II (ACR)	16.85 ± 0.47 <sup>cb</sup>	43.39 ± 1.34 <sup>da</sup>	39.03 ± 1.91 <sup>ca</sup>
Group III (Garlic)	30.51 ± 0.60 <sup>ac</sup>	125.48 ± 1.73 <sup>aa</sup>	83.39 ± 2.47 <sup>ab</sup>
Group IV (ACR+ Garlic)	26.97 ± 0.47 <sup>bc</sup>	83.61 ± 1.34 <sup>ba</sup>	53.11 ± 1.91 <sup>bb</sup>

\*Means within the same column and row carrying different letters are significantly different at (P<0.05), GSH= Glutathione, ACR= Acrylamide.

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