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Eliminating the toxic effect of acrylamide by garlic and mustard through the induction of antioxidant enzyme

Microbiology

KEYWORDS : Acrylamide, glutathione-s-transferase, garlic, mustard

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ABSTRACT

Acrylamide (ACR) is known to cause oxidative stress and other toxic effects in different tissues by reducing the activity of phase II biotransformation enzyme, Glutathione-S-Transferase (GST). Potent bioactive compounds from garlic and yellow mustard can reverse the adverse effects of ACR by enhancing the GST activity. Our in vitro study showed that GST activity increased in ACR-treated tissues, simultaneously supplemented with garlic and mustard extracts, both alone and in combination as compared to ACR-treated tissues without any supplementation in which GST activity was greatly reduced when compared to normal control group. Thus, we concluded that garlic and mustard are successful in averting the harmful effects induced by ACR, as reflected in elevated activity of antioxidant enzyme, Glutathione-S-Transferase.

INTRODUCTION

Acrylamide (ACR) is a reactive water soluble vinyl monomer [1] nowadays prepared commercially by the hydrolysis of acrylonitrile by nitrile hydratase [2]. Industrially, ACR is mostly used to synthesize polyacrylamide which is used extensively in water purification, ore processing, tertiary oil recovery, as a soil stabilizer,making of cosmetics, glues and paper, and for polyacrylamide gel electrophoresis (SDS-PAGE) in research based work [1]. ACR occurs as a toxicant in carbohydrate rich foods cooked at high temperature, produced through maillard reaction between amino acids, especially asparagine and reducing sugars such as glucose or fructose [1]. Non-thermal decomposition of ACRproduces ammonia whereas thermal decomposition produces carbon monoxide, carbon dioxide, and oxides of nitrogen [3]. Exposure to ACR has become a world wide concern after the publication of International Agency for Research on Cancer (IARC) in 1994, where it is stated that it is a probable human carcinogen [4]. In mice model, it has shown cancerous effect and nervous system damage [1]. It is considered to be hepatotoxic [1], neurotoxic [1] and produces gastric mucosal erosions and depletion of the protective surface mucus along with inflammation [5].

The effect of ACR on different biochemical enzymes has been studied both in vivo and in vitro [6]. In brain tissue of ACR-treated developing chick embryos, level of antioxidant enzyme Glutathione-S-Transferase (GST) significantly decreased at higher doses with increasing time length as compared to control groups [7]. Administration of acrylamide in rats also resulted in reduction in the level of GST in the liver, small intestine and large intestine respectively [8].GST enzymes found in organisms as diverse as microbes, insects, plants, fish and mammals [9] plays a major role in phase II drug metabolism by contributing to cell survival by detoxification of foreign compounds. These also participate in intracellular binding and transport of a broad range of lipophilic compounds including bilirubin, and hormones such as the glucocorticoids and thyroid hormones thus overall providing cellular defence against oxidative stress [10]. ACR may be conjugated by GST to form N-acetyl-S-(3-amino-3-oxopropyl) cysteine and excreted through urinary passage [11]. Thus increased GST activity may have a role in eliminating the toxic effect of ACR to great extent.

Recent years have seen an increasing emphasis on different foods, spices and their principle components in disease prevention. Bioactive compounds in Indian spices possess multiple beneficial effects such as anti-inflammatory, anti-microbial, anti-tumor etc. These effects are accomplished by modifying the activity of important cellular enzymes,thus giving protection against different ailments [12]. Two Indian spices garlic and mustard are known to contain potent organosulphur compounds that modulate the activity of GST enzyme, thereby, effectively reducing the risk of infection, inflammation, cancerous growth, cardiovascular diseases etc [12].In addition to being able to reach different organs of the body, certain bioactive compounds obtained from garlic and mustard extractsrespectively can cross the blood brain barrier effectively [13,14].

In vivo garlic alone or in combination with acrylamide causes significant increase in theactivity of GST in brain and liver tissue [1]. In vitro activity of GST enzyme was shown to increase in stomach tissue of mice in presence of both garlic and mustard extract, alone and in combination respectively as compared to the control groups [12].Similarly sulforaphane, obtained from mustard [15] is known to provide protection against neurodegenerative diseases by increasing the activity of GST [14].Allylisothiocyanate from mustard [16] induces phase II detoxifying enzyme GST in varied organs of the body, including liver and stomach [17].

The main purpose of this study was to assess the in vitro level and rate of activity of GST enzyme before and after treatment with acrylamide, in presence and absence of garlic and mustard extract, both individually and in combination in stomach, brain and liver tissues obtained from mice under normal condition.

MATERIALS AND METHODS

Chemicals

Acrylamide (99% purity) was purchased from Qualigens Fine Chemicals.1-chloro-2,4-dinitrobenzene (CDNB), Reduced Glutathione (GSH), phenyl methane sulfonyl fluoride (PMSF) were obtained from Sisco Research Laboratory Pvt. Ltd. (SRL). 2-mercaptoethanol from HIMEDIA. The other compounds and chemicals were readily available in the laboratory.

Experimental animal

BALB/c mice (male) were purchased from animal house of Indian Institute of Chemical Biology, Kolkata and were housed in cages at 28±2°C, at departmental animal house (Lady Brabourne College, Kolkata). They were fed with standard laboratory food and water.

The investigation conforms to the Guidelines for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). It was also approved by the Institutional Animal Ethics Committee, University of Calcutta (Registration no. 885/ac/05/CPCSEA), registered under Committee for the Purpose of Control and Supervision of Experiments on Laboratory Animals (CPCSEA), Minis-

try of Environment and Forests, Government of India.

The mice were sacrificed using mild chloroform anaesthesia and whole stomach, brain and liver from each mice were respectively excised and immediately kept on ice for processing further and use in the enzymatic assay.

Preparation of garlic and mustard extracts

Fresh raw garlic cloves were peeled, washed with distilled water and made into fine paste with sterile water. Garlic paste was then weighed and dissolved in sterile water at the ratio of 4:5 (wt/vol). Following the same method mustard paste was prepared at the ratio of 1:10 (wt/vol) containing 1% DMSO. Both were magnetically stirred for 2 hours separately. They were collected over filtration and then centrifuged for 15 minutes at 2000 rpm. The supernatants were collected separately for further use and final concentrations were determined which was 800 mg/ml for garlic extract and 100 mg/ml for mustard extract respectively [12].

Preparation of acrylamide

2 % (20 mg/ml) acrylamide was weighed and dissolved in a fixed volume of sterile distilled water.

Preparation of tissue homogenates

All the tissue samples (stomach, brain and liver) from mice were washed with cold PBS and each separately homogenized using a homogenizer with PBS containing 2-mercaptoethanol and PMSF. 10% of the tissue extract of each sample was taken separately with 90% of PBS buffer and centrifuged for 45 minutes at 4°C in a cooling centrifuge at 10000 rpm [12]. The supernatant of each sample was collected and utilized as whole enzyme source.

Preparation of experimental homogenates

The tissue collected were grouped as following and the experiment was conducted in triplicate.

For brain sample - i} B1 (Control) ii} B2 (Tissue with garlic extract, 1:1 ratio) iii} B3 (Tissue with mustard extract, 1:1 ratio) iv} B4 (Tissue with both garlic and mustard extract, 1:1:1 ratio) v} B5 (Tissue with acrylamide, 1:1 ratio) vi} B6 (Tissue with acrylamide and garlic extract, 1:1:1 ratio) vii} B7 (Tissue with acrylamide and mustard extract, 1:1:1 ratio) viii} B8 (Tissue with acrylamide, garlic and mustard extract, 1:1:1:1 ratio).

Similarly, stomach tissue and liver tissue were grouped from S1 to S8 and L1 to L8 respectively for the experiments.

Determination of GST activity and protein content

The prepared experimental homogenates were incubated at room temperature for three (3) hours, after which enzymatic activity for Glutathione-S-Transferase (GST) was measuredspectrophotometrically for each of the sample at 340 nm. following the standard methodology [18] with slight modifications.

The protein contents of the samples were quantified by standard Folin-Ciocalteau method. The absorbance was measured at 660 nm.

STATISTICAL ANALYSIS

The data was analysed using Standard Deviation formulae. The results were expressed as mean ± S.D of 3 mice for each group.

RESULTS AND DISCUSSION

The results given in Table 1 and Figure 1 indicate that the GST enzyme is active in vitro condition in different normal tissues obtained from mice, gave 29.35, 85.82 and 2.46 (ΔOD/mg protein/unit time) of specific activity for B1 (normal brain tissue control), S1 (normal stomach tissue control) and L1 (normal liver tissue control) respectively. The in vitro treatment of different tissues (brain, stomach and liver) with garlic (800 mg/ml) or mustard (100 mg/ml) extract alone or in combination significantly increases activity of GST enzyme as compared to the control tissue samples.

It also revealed that the tissue samples treated with acrylamide at 20 mg/ml, alone showed considerable reduction in the GST specific activity, 27.17 ΔOD/mg protein/unit time for B5 (brain tissue with acrylamide), 78.32 ΔOD/mg protein/unit time for S5 (stomach tissue with ACR) and 1.55 ΔOD/mg protein/unit time for L5 (liver tissue with ACR), that is lowest activities for each tissue sample when compared with all others but ACR-treated tissue homogenates when simultaneously incubated with garlic or mustard extracts or both in combination helped elevate GST activity in all the samples.

For the brain and liver samples respectively, GST activity was found to increase most in ACR-treated tissue incubated with garlic extract alone, from 27.17 to 33.48 (ΔOD/mg protein/unit time) in brain and 1.55 to 2.03 (ΔOD/mg protein/unit time) in liver.

In case of stomach tissue, GST specific activity increased the most in ACR-treated tissue incubated with both garlic and mustard extracts in combination, from 78.32 to 83.68 (ΔOD/mg protein/unit time).

These results concur with previous studies which reported a decrease in the activity of GST in brain [1,7], stomach [8] and liver [1] of mice on subsequent exposure to acrylamide. These observations also concur with those obtained previously who reported that, treatment with garlic [19] and mustard extracts [12] causes recovery of glutathione depletion with increased activity of antioxidant enzyme, GST, thus reducing oxidative stress. The present findings therefore, hold true for all the experiments conducted.

CONCLUSION

In conclusion, it can be inferred that acrylamide can cause many adverse effects in different tissues, both in vitro and in vivo, as is reflected in significant decrease in the activity of Glutathione-S-Transferase, which is an important phase II biotransformation enzyme involved in the removal of xenobiotics from the body. Simultaneous administration of garlic or mustard alone and in combination, to ACR-treated tissue effectively enhances the activity of GST thereby, agreeing with previous reports that the protective effects of garlic and mustard extracts involves maintenance of antioxidant capacity in preventing the tissues against oxidative stress. It is also reported that acrylamide is formed as a toxicant in potatoes based products cooked at high temperature, such as chips, so consumption of these may induce acrylamide toxicity in human as well [1]. Thus, this in vitro observation on mice can be extrapolated to human cases, suffering from ACR toxicity,for in vivo study, by further research, by administration of beneficial spices.

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Table 1: Average (Mean) specific activity of GST enzyme in tissue samples at 340 nm (ΔOD/mg protein/unit time)

Brain samples (Mean±SD)		Stomach samples (Mean±SD)		Liver samples (Mean±SD)	
B1	29.35±1.93	S1	85.82±1.91	L1	2.46±0.36
B2	31.13±1.67	S2	85.91±3.32	L2	2.48±0.44
B3	37.30±2.04*	S3	82.05±1.74	L3	2.49±0.42
B4	35±1.15*	S4	85±1.80	L4	2.52±0.42
B5	27.17±1.91	S5	78.32±1.68*	L5	1.55±0.09*
B6	33.70±1.33*[a]	S6	81.05±1.14*	L6	2.03±0.23
B7	32.48±2.75	S7	82.36±1.54[b]	L7	1.67±0.43
B8	29.87±0.49	S8	83.68±0.88[b]	L8	1.99±0.03[c]

n = 3 for each group (each individual sample), values significantly different compared to control group, *p<0.05.

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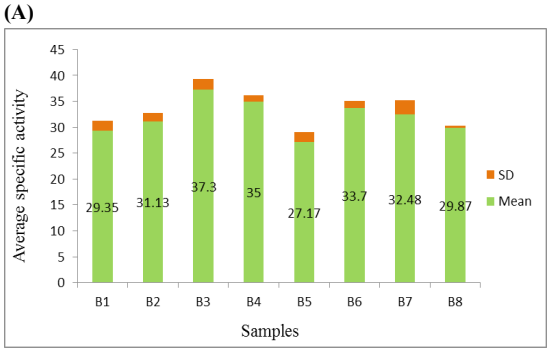
a = values significantly different compared to B5, $p<0.05$. b = values significantly different compared to S5, $p<0.05$. c = values significantly different compared to L5, $p<0.05$.

For brain sample - i} B1 (Control) ii} B2 (Tissue with garlic extract) iii} B3 (Tissue with mustard extract) iv} B4 (Tissue with both garlic and mustard extract) v} B5 (Tissue with acrylamide) vi} B6 (Tissue with acrylamide and garlic extract) vii} B7 (Tissue with acrylamide and mustard extract) viii} B8 (Tissue with acrylamide, garlic and mustard extract).

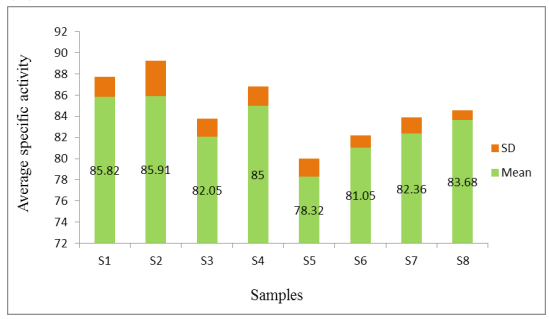
Similarly, stomach tissue and liver tissue were grouped from S1 to S8 and L1 to L8 respectively.

Figure 1: Average (Mean) specific activity of GST enzyme at 340 nm (ΔOD/mg protein/unit time) in (A) Brain (B) Stomach (C) Liver

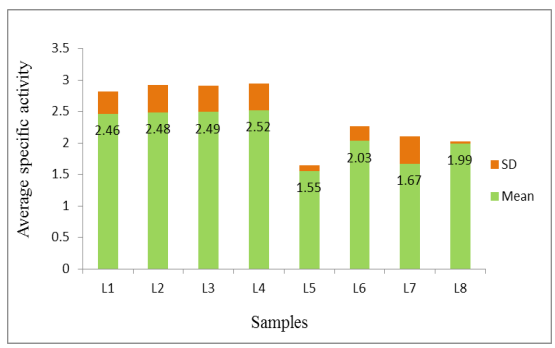
(A)



(B)



(C)



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