Int. J. Exp. Res. Rev., Vol. 6: 1-11 (2016)

International Journal of Experimental Research and Review (IJERR)
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ISSN: 2455-4855 (Online)

Original Article

Received: 2nd June, 2016; **Accepted:** 3rd July, 2016; **Published:** 30th August, 2016

Remedial effects of aged Garlic extract during acute and chronic arsenic exposure in mice

Tapan Kumar Pal* and Sachi Nandan Kundu

Department of Zoology, University of Kalyani, Kalyani - 741 235, West Bengal, India.

***Corresponding author:** taponiaindica@gmail.com

Abstract

Regular oral supplementation of aged Garlic (*Allium sativum* L.) extract during acute (5mg/kg body weight at a time, intraperitoneally, for five days) and chronic arsenic exposures (0.2 mg/kg body weight, intraperitoneally, weekly for 12 weeks) to Swiss albino mice was investigated. Bradford assay and gel electrophoresis study exhibited that the total protein contents in some tissues had decreased by the arsenic toxicity, but revived by garlic supplementation. *In vitro* study revealed that the inhibition of the activities of some enzymes like lipid peroxidase, acid phosphatase and alkaline phosphatase, extracted from arsenic-treated liver, kidney, spleen and thymus tissues was reversed by the incubation of the said tissues with garlic extract. The atomic absorption spectrometric study displayed that the arsenic accumulation in garlic-fed mice was much less compared to that in unfed mice. These results elucidated the promising role of garlic against arsenic toxicity.

Keywords: Acute and chronic toxicity, aged garlic extract, arsenic trioxide, mice.

Introduction

Since last century billions of people get affected even died due to consumption of Arsenic contaminated drinking water. The poison king Arsenic causes tremendous health hazards like keratological lesion, skin cancer, black foot disease, reproductive failure, cerebro-vascular disease, gastro intestinal discomfort, polyneuropathy, organ failure like kidney, liver, lungs etc even death (Neiger and Osweiler, 1989; Jollife et al., 1991; Dong et al., 1993; Chiou et al., 1995). Among several root of arsenic exposure, natural mode, i.e. via drinking water, is very common rather than anthropogenic or industrial exposure. As per WHO's recommendation, in the arsenic-prone countries like India, Bangladesh, etc, the permissible limit for the concentration of arsenic in drinking water is 50µg L^{-1} (i.e., 50 parts per billion), whereas for the rest of the world, the limit is 10 ppb (Vaughan, 2006). The approved clinical measure for arsenic affected one is chelation therapy. Some chelating agents are used to scavenge toxic arsenic and convert it to a non-toxic form. British Anti Lewisite (BAL; 2,3-dimercaprol), a dithiol compound having a strong chelating affinity for arsenic, was used as the drug of choice for long days (Snider et al.,

1990; Muckter et al., 1997). But, due to side effects like mucosal burning sensations, increase in blood pressure, nausea and vomiting, headache, conjunctivitis, lacrimation and chest pain (Vilensky and Redman, 2003; Wang et al., 2007) BAL is not presently used in treatment. Rather modified versions of BAL [meso 2, 3-dimercaptosuccinic acid (DMSA) and 2, 3-dimercaptopropane 1-sulfonate (DMPS)] are now the choice for treatment, either alone or in combination with vitamin C, vitamin E or Zinc (Ramanathan et al., 2002; Kannan and Flora, 2004; Modi, 2005, 2006). Yet, low therapeutic index and some adverse effects of these drugs indicate, chemical metal chelator should not be the only clinical choice, rather natural compounds available in our common food stuff or in common herb can be helpful too. One such a common herb as well as food stuff is- Garlic (*Allium sativum* L., Food of Gods). Several ancient world literatures like Atharva Veda, Charak Samhita, Sushruta Samhita etc. even different tribal and non-tribal folk and some traditional knowledge describe several medicinal values of garlic. Several clinical investigations prove garlic as a potent anti-oxidant, which is used to treat cancer too. Garlic protects DNA against free radical-mediated damage and mutations, inhibits multistep carcinogenesis and defends against ionizing radiation and UV-induced damage (Borek, 2001). From several other promising research the present study is inspired and focused to investigate whether aged garlic extract may help to protect induced chronic arsenic toxicity at cellular level by reducing oxidative stress or eliminate arsenic as a nontoxic form from body. Here fresh garlic extracts are allowed aging for a long period to modify unstable molecules (allin, allicin, etc.) to a stable more potent antioxidant and increasing highly bioavailable water-soluble organosulfur compounds, such as *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC) (Imai et al., 1994).

Materials and Methods

Animals

Inbred strain of Swiss albino mice (*Mus musculus*) were used by maintaining them in the animal house facility of the Department of Zoology, Kalyani University, with due permission of the Animal Welfare Committee regarding ethical issues. Healthy mice of 18-20g, reared for 12 weeks with 12 hour light : dark cycle at temperature 23 (±2)^{0}C and humidity 55 (±5)%, were provided with pellet diet of West Bengal Dairy & Poultry Development Corporation Ltd., Kalyani, Nadia and filtered tap water *ad libitum*.

Garlic extract (4% w/v stock) preparation

Aged garlic extract (AGE) was prepared according to the method of Kasuga et al., 2001, with partial modification. Matured garlic (*Allium sativum* L. Family-Alliaceae, purchased from local market) cloves/bulbs were first smashed by mortar and pestle. The paste was then suspended in water and kept overnight. On the next day, the suspension was heated at 80^{0}C in a water bath for 15 minutes. The extract was subsequently filtered through 0.45 micron whatmann paper. The filtrate, for aging, was stored at -20^{0}C for one month.

Arsenic trioxide solution preparation

A stock solution (100 mg/mL) was prepared by dissolving As_2O_3 (Sigma) in milli-Q water by stirring magnetically with moderate heating.

Experimental groups and treatment

ATO was administered intra-peritoneally (ip) for chronic and acute exposure at a dose of 0.2mg/kg body weight (b/w) per week for 12 weeks and 5 mg/kg body weight (b/w)/day for

consecutive 5 days respectively. The mice were fed AGE with water in such a way that a dose of (100mg/kg b/w) was consumed by them; each mouse was found to consume (5 ± 2mL) water per day. Experiments were performed on following groups–Group-I (A): ATO (ip), Group-II (AG): ATO (ip) + Aqueous AGE, Group-III (G): Distilled water [D/W] (ip) + Aqueous AGE, Group-IV (C): D/W (ip).

Sampling

Tissue and enzyme

Mice of both control and treated groups were sacrificed under light ether anesthesia. Liver, kidney, spleen and thymus tissues were dissected, washed with ice-cold 0.9% normal saline, blotted dry and stored in ice chamber until analysis.

To obtain the cell extract, a stored tissue was first homogenized and was subsequently centrifuged at 10,000 rpm for 10 min. The supernatant was finally collected as the cell extract, which was used as the source of the enzyme.

Experiments

***In vitro* enzymatic assay**

For this study different organs (liver, kidney, spleen and thymus) of mice from control group (Gr-C) were used. Experiments were done in test tubes (inspired by Tabassum, 2006). For this, four groups were set up, **Group-I**: ATO incubated tissues; **Group-II**: ATO+AGE incubated tissues; **Group-III**: tissues with AGE incubation and **Group-IV:** tissues without any incubation. Isolated tissues (100mg each) were incubated with 100ppm ATO (1mL) at 37^0C for 45 minutes. After washing with physiological saline (two times) the tissues were again incubated for another 45 minutes at 37^0C with 100µl AGE stock (1mL). After washing with physiological saline (three times) the tissues were then used for assaying alkaline phosphatase, acid phosphatase and lipid peroxidase activity as described below.

Alkaline and Acid Phosphatase activity

Enzymatic activity of alkaline and acid phosphatases was assayed by the method of Walter and Schutt (1974), where p-nitrophenyl-phosphate and p-nitrophenol were respectively the substrate and the product of the enzymatic reaction. The amount of p-nitrophenol liberated was proportional to the enzymatic activity and was measured in a spectrophotometer (VARIAN CARY50 BIO) at 405 nm. The molar extinction coefficient (1.78×10^4 M^{-1} cm^{-1}) of p-nitrophenol was used (Zhang and Van Etten, 1991) to find out its concentration from Beer-Lambert law.

Lipid Peroxidase activity

Oxidative stress in the cellular environment results in the formation of highly reactive and unstable lipid hydro-peroxides, decomposition of which by lipid peroxidase results in the formation of malondialdehyde (MDA). The MDA can be quantified colorimetrically following its controlled reaction with thiobarbituric acid (Wilber et al., 1949). Briefly, tissues were homogenized in 1.5% KCl. The homogenate (1 mL) was incubated with 1.0 mL of 20% trichloroacetic acid. Then, the reaction mixture was incubated with 1.0mL of 0.67% (w/v) thiobarbituric acid for 15minutes in a boiling water bath. The pink chromogen formed was centrifuged (10,000 rpm for 10 minutes) and the supernatant collected for spectroscopic assay at 535nm in VARIAN CARY50 BIO spectrometer. The lipid peroxidase activity was proportional to the amount of chromogen produced, which was calculated from its molar extinction coefficient of 1.56 X 105 M^{-1} cm^{-1} (Lovell et al., 1995).

Protein estimation

Proteins were estimated by the method of Bradford (1976), using BSA as the standard protein.

Gel electrophoresis by SDS-PAGE

The protein samples were electrophoresed in 12% SDS–polyacrylamide and protein bands were stained with 0.25% Coomassie brilliant blue (R-250) staining solution. Protein bands were analyzed by ImageJ software for densitometric study (Laemmli, 1970).

Arsenic estimation

Inorganic arsenic concentration in liver, kidney, spleen, and thymus were measured by using atomic absorption spectrophotometer. For this, tissues were digested first by a mixture of H_2SO_4 and HNO_3 (1:2) in borosilicate flask for 2-3 hr at 80^0C on a sand bath. To 1.0mL of this digested sample, 1.0mL concentrated HCl, 1.0 mL 5% (w/v) KI and 5% (w/v) ascorbic acid were added one after another for reduction of As (V) to As (III). After incubating for 45 minutes at ambient temperature, the sample was diluted to 10mL by adding D/W. For hydride generation, 0.2% (w/v) $NaBH_4$ in 0.05% (w/v) NaOH was used. As the carrier solution 10% (v/v) HCl and as the carrier gas argon was used. Finally the arsenic content was analyzed using an atomic absorption spectrometer (Perkin Elmer, Analyst 100), interfaced with FIAS 100 flow injection hydride generator.

Statistical analysis

Data are expressed as mean ± SEM. The test of significance was analyzed by student's t-test and one way ANOVA. P value <0.05 was considered as significant. Graph Pad In Stat-3 statistical software package was used for this purpose.

Results

Physical and Behavioural

ATO-treated mice (Gr-A) did not show any abnormal behavior except drowsiness. Both acute and chronic study showed (Table-1 and 2) decrease in body weight (by 39.75 and 24.4% respectively after 12th week) in arsenic treated group. For AG-group mice, there was also decrease in body weight, but the extent of decrease was less (about 28.41 and 16.4% after 12 weeks in acute and chronic study). No decrease in weight was observed for G-group mice. Therefore, the loss in body weight by the arsenic action was partly reverted by the action of AGE. The percentage of AGE-mediated protection in arsenic-mediated loss in body weight was 28.5% and 32.8% in acute and chronic study respectively. The calculation of % benefit was done by-[{(Loss in b. w.)$_{A\ group}$ – (Loss in b. w.)$_{AG\ group}$} / (Loss in b. w.)$_{A\ group}$] X 100.

Reddish wart/rash was found on mouth, head, pinna, abdomen, limbs and tail of Gr-A animals; no such rash was observed in any other groups of mice, implying that the AGE neutralized the adverse effect (physical symptoms) of arsenic.

Biochemical

In this sub-section, the results in activity of different enzymes like alkaline phosphatase, acid phosphatase and lipid peroxidase in different organs like liver, kidney, spleen and thymus of the mice of four different groups I, II, III and IV have been presented.

Alkaline Phosphatase (ALP)

The activity of ALP in liver(Fig-1a) of Gr-I was found to be significantly 350.14% higher than Gr-IV. Gr-III showed significantly 38.87% higher ALP activity than Gr-IV. Although Gr-II showed

significantly lesser ALP activity than Gr-I, an overall 14.26% benefit was available in Gr-II.

Not only in liver, the activity of ALP also increased in kidney, spleen and thymus and in these organs the average percent protection of AGE on arsenic stress was about 34, 19 and 38%

Table 1. Body weight (gram) in chronic study.

Group	2nd week	4th week	6th week	8th week	10th week	12th week
A	20.2±0.6042*	22.7±1.168	23.1±1.042	25.1±1.042	25.3±0.7176α	26.3±0.7176β
AG	20.2±0.7842	23.4±0.7314	23.9±1.017	26.8±0.515	29.4±0.5788a	29.1±0.5788*β
G	20.9±0.8276	22.5±0.8216	24.5±0.9354	26.6±1.017	30.8±0.8155b	35.7±0.6442b
C	19.80±0.5148	24.4±0.6964	23.8±0.7176	26.9±0.332	29.8±0.8155a	34.8±0.6042b

Table 2. Body weight (gram) in acute study.

Group	2nd week	4th week	6th week	8th week	10th week	12th week
A	18.2±1.032	17.9±0.914β	20.3±0.464α	22.1±0.292β	23.7±0.561β	24.9±0.62 β
AG	18.4±0.857	20.4±0.62	20.9±0.992	24.4±0.367aβ	25.7±0.903†	27.1±0.828†
G	20.9±0.8276	22.5±0.8216a	24.5±0.9354a	26.6±1.017a	30.8±0.8155b	35.7±0.6442b
C	19.80±0.5148	24.4±0.6964b	23.8±0.7176a	26.9±0.332b	29.8±0.8155b	34.8±0.6042b

Table 3. Protein content (µg protein/g tissue) in Liver (acute study).

Group	2nd week	4th week	6th week	8th week	10th week	12th week
A	149.2±1.158β	154±1.673 β	155.2±2.518β	162.4±2.502β	165.4±1.631β	168.4±3.124β
AG	156.4±1.72aβ	159.6±1.563*β	164.2±2.267*β	171.6±3.059*β	177.6±3.682*β	178±2.793β
G	176.4±0.872	182.4±1.208b	184.8±0.917b	185.2±1.2b	197.6±1.435b	198.6±1.364b
C	177.4±1.03b	184±1.225b	189.4±1.435b	187.6±1.691b	194±0.949b	198±1.304b

Table 4. Protein content (µg protein/g tissue) in Liver (chronic study).

Group	2nd week	4th week	6th week	8th week	10th week	12th week
A	173.4±1.03†	175±1.304α	174.6±1.568β	169.2±1.428β	166.4±0.872β	164.2±2.267β
AG	172±0.894	176.8±1.393	176.4±0.927β	170.4±1.503β	172±2.214+	177.2±1.158βb
G	176.4±0.872	182.4±1.208	184.8±0.917b	185.2±1.2b	197.6±1.435b	198.6±1.364b
C	177.4±1.03*	184±1.225a	189.4±1.435b	187.6±1.691b	194±0.949b	198±1.304b

Table 5. Arsenic accumulation (µg Arsenic/g wet tissue) in Liver during acute exposure (high dose) of ATO.

Group	2nd week	4th week	6th week	8th week	10th week	12th week
A	0.436±0.055β	0.82±0.037β	0.958±0.043β	1.498±0.037β	1.93±0.024β	2.51±0.056β
AG	0.33±0.03β	0.476±0.021bβ	0.528±0.033βb	0.672±0.034βb	0.736±0.031βb	0.90±0.074βb
G	0.068±0.005b	0.068±0.005b	0.068±0.005b	0.068±0.005b	0.068±0.005b	0.068±0.005b
C	0.073±0.005b	0.073±0.005b	0.073±0.005b	0.073±0.005b	0.073±0.005b	0.073±0.005b

Table 6. Arsenic accumulation (µg Arsenic/g wet tissue) in Liver during chronic exposure (low dose) of ATO.

Group	1st week	2nd week	4th week	6th week	8th week	10th week	12th week
A	35.22±0.878β	34.14±0.581β	30.042±0.853β	28.13±0.894β	25.79±1.016β	22.606±1.089β	20.02±0.499β
AG	29.84±1.399*β	24.648±0.972bβ	18.862±1.56bβ	16.184±1.007bβ	13.326±1.303bβ	9.234±0.737bβ	6.228±0.338bβ
G	0.068±0.005b	0.068±0.005b	0.068±0.005b	0.068±0.005b	0.068±0.005b	0.068±0.005b	0.068±0.005b
C	0.073±0.005b	0.073±0.005b	0.073±0.005b	0.073±0.005b	0.073±0.005b	0.073±0.005b	0.073±0.005b

Table 1 to Table-6, values are mean ± SEM; *n*=5. The symbols *****, **a** and **b** denote statistically significant values compared with group-A where ***** represent $p<0.05$, **a** represent $p<0.01$ and **b** represent $p<0.001$. Similarly, the symbols **†**, **α** and **β** denote statistically significant values compared with group-C where **†** represent $p<0.05$, **α** represent $p<0.01$ and **β** represent $p<0.001$.

respectively. Here percent benefit means AGE mediated restriction of higher enzymatic activity found during this assay is shown below and the calculation was done as: [{(Enzyme activity of Gr-I–Enzyme activity of Gr-IV)–(Enzyme activity of Gr-II–Enzyme activity of Gr-IV)}/(Enzyme activity of Gr-I–Enzyme activity of Gr-IV)] X 100. This ALP study implies clearly that the ALP activity has been enhanced in the presence of ATO (Because the possibility of enhancement of the amount of ALP in the tissue, during the 45 minutes tenure of ATO exposure was remote).

Acid Phosphatase (ACP)

The activity of ACP in liver (Fig-1b)of Gr-I was found to have significantly 79.76% higher than Gr-IV. Although Gr-II showed significantly lesser ACP activity (16.07%) than Gr-I, an overall 36.22% benefit (calculated as described in sub-section ALP) was available in Gr-II. No significant difference in enzymatic activity was found between Gr-III and Gr-IV. Like liver, the arsenic-induced increase in ACP activity was found in kidney, spleen and thymus too and in these organs, the AGE-mediated benefit of arsenic stress was found to be about 42, 53, and 57% respectively.

Lipid Peroxidase (LPO)

The LPO activity was measured by the amount of MDA liberated during enzymatic reaction. The activity of LPO in liver (Fig-1c) of Gr-I was found to be significantly 127.92% higher than Gr-IV. Although Gr-II showed significantly lesser LPO activity (21.95%) than Gr-I, an overall 39% benefit (calculated as described in sub-section ALP) was available in Gr-II. No significant difference of enzymatic activity was found between Gr-III and Gr-IV. Similarly, in other organs, like kidney, spleen and thymus also the AGE-mediated remedial benefit was measured to be about 50, 70 and 49% respectively. Enzymatic activities of Gr-IV tissue didn't show any significant difference than Gr-III tissue for LPO activity.

Protein estimation

The amount of total protein content in the respective experimental group has been shown in the Table 3 & 4 and analyzed statistically.

Chronic exposure

The total protein decreased gradually from 2nd week onwards reaching a maximum level on 12th week in Gr-A when compared with Gr-C. In AG-group the protein content was found to be maximum 7.91% higher than Gr-A and there was an average 22% protection (calculated similarly as described in section physical and Behavioural, here body weight should replaced by protein content) found from protein deterioration. There was no significant difference between Gr-G and Gr-C. A similar pattern of AGE mediated protection against protein deterioration observed in kidney, spleen and thymus tissue and the values were 33, 26 and 1% respectively.

Acute exposure

Amount of total protein content was also found to decrease since 2nd week but a natural revival mechanism helps to restrict further protein deterioration to some extent up to 12th week. But a better recovery was observed in garlic-fed group. Here an average recovery found at a 12 week stretch in liver, kidney, spleen and thymus are approximately 32, 19, 26 and 31 percent respectively.

Gel electrophoresis study

The band pattern of proteins of different molecular weight has been found to show low or high intensity indicating either due to protein

deterioration or higher level of protein expression. Both in chronic and acute exposure of ATO showed almost similar pattern of bands. Densitometric data of liver tissue has given in table 7.

AAS assay for Arsenic

Chronic exposure

In all the tissues (liver, kidney, spleen and thymus) of Gr-A and Gr-AG mice, there were gradual accumulation of inorganic arsenic with

Table 7. Densitometric analysis of band intensity for Liver tissue.

BAND	KDa (Approx.)	L1(A)	L2(AG)	L3(G)	L4(C)
B1	110	1.19	0.7	0.94	1
B2	95	0.38	0.82	0.95	1
B3	72	0.89	0.84	0.99	1
B4	70	0.67	0.71	0.87	1
B5	66	0.38	0.7	0.95	1
B6	60	0.36	0.77	0.97	1
B7	55	0.46	0.74	0.97	1
B8	50	0.38	0.77	0.95	1
B9	48	0.39	0.8	0.95	1
B10	46	0.76	0.76	0.97	1
B11	40	0.34	0.71	0.87	1
B12	34	1.0	1.05	1.13	1
B13	32	0.41	0.84	0.98	1
B14	30	0.42	0.85	1.1	1
B15	28	0.28	0.7	1.02	1
B16	25	0.22	0.62	0.84	1
B17	22	1.08	71	1.05	1
B18	17	0.4	0.84	0.92	1
B19	10	0.2	0.42	0.99	1
B20	7	1.09	0.88	1.08	1

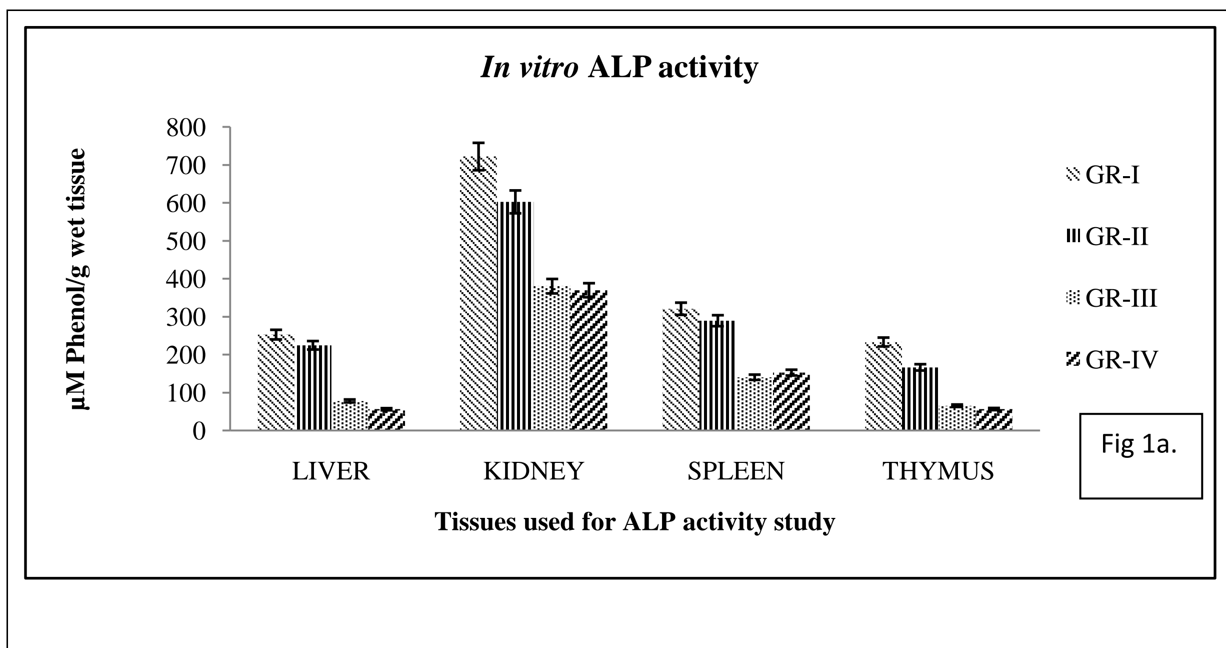

Fig 1a.

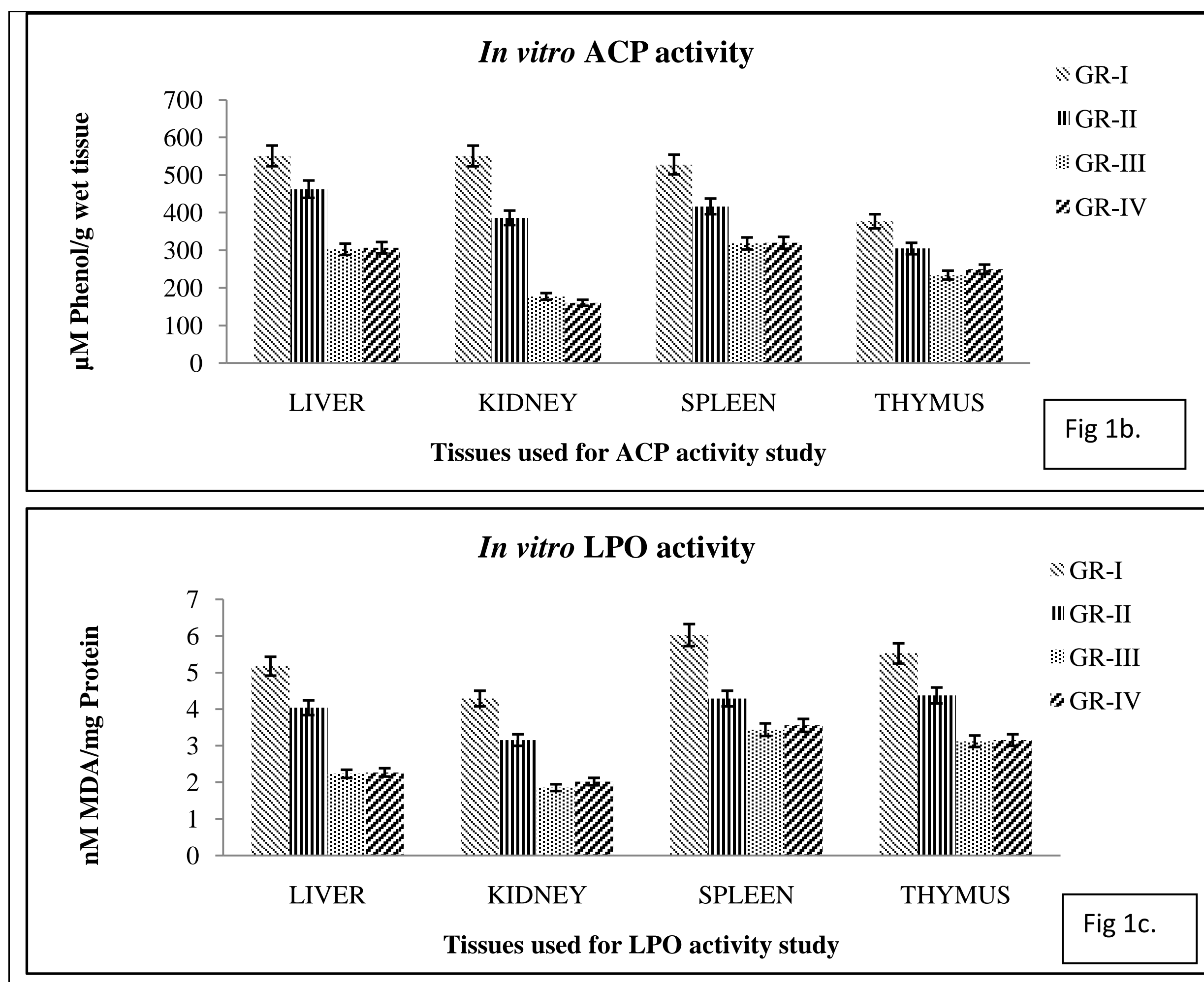

Fig 1(a, b & c). Variation of the *in vitro* activity of different enzymes of different groups [Gr-I (ATO-treated), Gr-II (ATO+AGE treated) Gr-III (AGE-treated) and Gr-IV (control tissue)]. Values are mean ± SEM; n=5. Here p<0.05.

weeks of arsenic exposure; however, in the said tissues, the arsenic retention in Gr-AG mice was significantly less than that in Gr-A mice (Table-6). From 2nd to 12th weeks of arsenic exposure, the average percentage of amelioration of arsenic damage in liver, kidney, spleen and thymus tissues was about 49, 45, 55 and 42% respectively. The maximum arsenic accumulation was found in spleen.

Acute exposure

Results from AAS data shows that tissues (liver, kidney, spleen and thymus) of Gr-A were found to have accumulated inorganic arsenic gradually in increasing amount than control (Gr-C). Arsenic content in all the tissues was found (Table-5) to be significantly lesser in Gr-AG than Gr-A mice consistently since 1st week onwards. In Gr-AG, average accumulation of arsenic was found to be lesser than Gr-A by 43% in liver, 40% in kidney, 21% in spleen and 29% in thymus. Spleen was found to have maximum arsenic accumulation than the other tissues assayed.

Comparing both the cases it was observed that rate of arsenic removal due to AGE per week in AG-group was considerably so high in acute arsenic exposed tissues (e.g., 0.701µg/g

wet liver tissue) than chronically arsenic exposed tissues (e.g., 0.125μg/g wet liver tissue) [Calculated as- {(amount of arsenic accumulation in 12th week in A-group - amount of arsenic accumulation in 1st week in A-group) - (amount of arsenic accumulation in 12th week in AG-group - amount of arsenic accumulation in 1st week in AG-group)}/12].

Discussion

From different biochemical and analytical studies, we report here that aged garlic extract partially but considerably reversed the acute as well as chronic ATO-induced toxicity in mouse model. From large number of research data it is well known that *Allium sativum* is a potent antioxidant combating with several kinds of stress at cellular level. AGE contains a rich source of flavonoids, unique water- and lipid-soluble organosulfur compounds (mostly chelating agents), and trace elements like selenium, tellurium etc. (Borek, 2001). In the present study few oxidative stress sensitive enzymes like phosphatase, peroxidase were assayed to observe their activity during ATO exposure, because arsenic was known to produce several reactive oxygen species. Reduced inorganic arsenic [As (III)] reacts strongly with sulfhydryl groups of proteins and thus alters function of many enzymes; moreover, arsenic also replaces phosphate group of various enzymes by itself (Hughes, 2002). In our earlier *in vivo* study (unpublished) it was found that the activity of the enzymes phosphatase and peroxidase had increased in ATO-treated mice and the similar indication was found in the present *in vitro* work. The increase in the activity of the enzymes might be due to the interaction of arsenic with the sulphur of the cysteine residues present in enzymes. Here, all the *in vitro* studies were based on control animal tissues to establish only the effect of ATO on enzymatic activity and to nullify all other internal factors which could have the possibility to arise in the *in vivo* study. This *in vitro* result also helps to establish the role of AGE combating against ATO toxicity. Thus our result strongly supports our logic. The indication of protein deterioration also implies ATO toxicity of protein metabolism in both acute and chronic study. Protein restoration found in garlic fed mice strongly support the beneficial role of AGE against arsenic toxicity. Although SDS-PAGE data does not help us to conclude any argue.

From AAS study it could also be predicted that AGE showed its efficacy by reducing arsenic load in tissue; the possible reason behind this was the chelation of arsenic by sulfur compounds of garlic. The reduction in arsenic load further implied that the AGE-chelated arsenic was finally removed from the tissues. The higher rate of arsenic removal in acute study also indicates AGE can effectively remove accumulated arsenic from body rather free arsenic. The highest accumulation of arsenic in spleen, as observed from our result, might be justified as the spleen, being the site of destruction of blood cells, contained higher amount of arsenic contributed by the arsenic accumulated blood cells.

The proposed mechanism of arsenic metabolism was methylation of arsenic (III) and its subsequent release in inactive, non-toxic form (Vahter, 1994; Challenger, 1945). Besides, our study indicated that the presence of several sulfur compounds (electronegative moiety) in AGE might help to detoxify arsenic (electropositive element) by interacting with it and thus did not permit binding of arsenic to sulfur and phosphorus moieties of amino acids of protein. The chelation of sodium arsenite by garlic compounds was evident from earlier *in vitro* study (Chowdhury et al., 2008). AGE

contains natural selenium which is a known antioxidant and was found to bind with arsenic forming arsenic-hemiselenide compound (Berry and Galle, 1994 and Kenyon et al., 1997) or Seleno-bis (S-glutathionyl) arsinium ion (Gailer et al., 2000); therefore, other than sulfur compounds, the selenium in AGE could also reduce the availability of free arsenic ions in the mice body.

Finally, it can be said that AGE must have remedial role combating against acute and chronic ATO mediated toxicity.

Acknowledgement

We acknowledge Prof. S.C. Santra (Department of Environmental Science, University of Kalyani) for accessing AAS facility.

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