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HOW ANTIOXIDANT AND ANTIOXIDANT ENZYME FUNCTIONS DO SUPPRESSED IN THE BRAIN TISSUES OF DEVELOPING CHICK EMBRYO UNDER THE INFLUENCE OF ACRYLAMIDE?

M. VENKATASWAMY, M. MEENABAI[1], K. VENKATASUBBAIAH[2], K. J. RAO[2] AND K. THYAGA RAJU*
[1]Department of Biochemistry (DST FIST and UGC BSR), Sri Venkateswara University, Tirupati - 517 502
[2]Department of Zoology, Sri Venkateswara University, Tirupati - 517 502, A. P., INDIA
e-mail: thyagarajuk_2002@rediffmail.com

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***Corresponding author**

ABSTRACT
Acrylamide a known neurotoxin is produced due to amino acid metabolism in the body. This molecule adversely affects the central nervous system by the operation of the free radicals and oxidative stress. These oxygenated molecules potential has been studied in brain tissue of day (11) (d11) developing chick embryo exposed to acrylamide at different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6mg) and times of intervals (24, 48 and 72h). Upon exposure of chick embryo to acrylamide the level of lipid peroxidation (LPO) product, malondialdehyde (MDA) increased and the contents of antioxidants i.e., reduced glutathione (GSH) and ascorbic acid (Vit-C) was decreased, significantly, in brain. The activities of antioxidant enzymes, glutathione-S-transferases (GSTs) and superoxide dismutase (SOD) have been increased up to 0.4mg and declined from 0.5 to 0.6mg due to dose and time dependant of acrylamide treatment and the activities of glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) were decreased. The present data conclude that acrylamide as neurotoxicant induces oxidative stress in developing chick embryo brain due to increasing rate of lipid peroxidation and suppress ion of the antioxidant enzymes and non-enzymatic antioxidant defense mechanism. These effects may provide an evidence for acrylamide induced neuronal damage in developing chick embryo up on the suppression and reduction of oxygen radical limiting enzymes.

INTRODUCTION

Acrylamide (AC) ($CH_2=CH\_CONH_2$), is used worldwide to synthesize polyacrylamide. Polyacrylamide has found numerous applications as a soil conditioner, in wastewater treatment, in the cosmetic, paper and textile industries and in the laboratory as a solid support for the separation of proteins and nucleic acids by electrophoresis (WHO, 1985; Gold and Schaumburg, 2000; LoPachin *et al*., 2003). Recently, discovery of acrylamide in a variety of human foods was reported by Swedish researchers (Tareke *et al*., 2000 and 2004) Acrylamide exposure by organisms has been associated with signs of impaired neurological performance in central and peripheral nervous systems that include impaired motor function and muscle weakness (Dumitru, 1989; Mulloy, 1996; Gjerlÿff *et al*., 2001). Human data that is available from cross-sectional studies included self-reported symptoms and neurological evaluations of acrylamide exposed workers with potential for inhalation and dermal (and possibly oral) exposure (Myers *et al*., 1991; Calleman *et al*., 1994; Hagmar *et al*., 2001) provide a supportive evidence of acrylamide-induced neurotoxicity, however they lack information regarding relative contributions of natural exposure routes (inhalation, oral, dermal), exposure-response relationships and other confounding exposures.

Acrylamide has been extensively investigated and has a large database of very complex toxicity, pharmacokinetic and mode of action studies. The results of the animal toxicity studies indicate that acrylamide is carcinogenic in rodents and produces toxic effects on the reproductive and nervous systems. However, to date, only neurotoxicity has been demonstrated in humans. Multiple studies have been conducted to assess neurotoxic effects of acrylamide in occupationally-exposed workers in small factories manufacturing acrylamide in China (He *et al*., 1989; Deng *et al*., 1993) and South Africa (Myers *et al*., 1991). AC is a well-documented neurotoxicant in both humans and laboratory animals. Subchronic, low-level occupational exposure of human produces neurotoxicity characterized by ataxia, skeletal muscle weakness, and numbness of the hands and feet (He *et al*., 1989; Deng *et al*., 1993). The neurotoxicity of AC has been extensively studied with respect to mammalian species including mice, rats, guinea pigs, cats, dogs and monkeys at daily dose rates varying from 0.1 to 50 mg/kg/day (LoPachin and Lehning, 1994; LoPachin *et al*., 2000). Early morphologic studies revealed that low-dose subchronic induction of AC neurotoxicity was associated with nerve damage in both the central and peripheral nervous systems (LoPachin *et al*., 2002). On the basis of evidence of early structural and functional damage, LoPachin *et al*., 2002 suggested that neuron ends were the primary targets of AC action and that synaptic dysfunction and subsequent degeneration were sufficient steps for production of AC neurotoxicity.

The organisms have developed antioxidant defense in order to minimize oxidative damage to cellular components as lipids,

proteins and DNA. Reactive oxygen species (ROS) are the most studied biomarkers to evaluate the biochemical alterations of organic contaminants on aquatic organisms. ROS include superoxide anion radical (O_2), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH). The most important antioxidant enzymes which are involved in the elimination of ROS include superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and glutathione peroxidase (GPx, EC 1.11.1.9). The other group of enzymes which involved indirectly was Glutathione S-transferases (GST, EC 2.5.1.18) which catalyses the conjugation of glutathione (GSH) using various electrophilic substances and play a role in prevention of oxidative damage. Hence forth organisms can adapt itself for the elimination of oxygen species to the increasing ROS production by up-regulating antioxidant enzymes (Livingstone, 2003). Failure of defense to detoxify the excess of ROS production can lead to significant oxidative damage including enzyme inactivation, protein and DNA and lipid degradation (Di Giulio *et al*., 2008). Therefore considering the significance of acrylamide induced neurotoxicity in developing chick embryo brain, the aim of the present work was to assess the effects of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6mg acrylamide concentrations on oxidative stress and oxidative enzymes.

The brain may exhibit distinct variations in cellular as well as regional distribution of antioxidant biochemical defenses (Ansari *et al*., 1989; Ravindranath *et al*., 1989; Verma *et al*., 2001). Thus, neural cells and/or brain regions likely differentially respond to changes in metabolic rates associated with the generation of ROS (Hussain *et al*., 1995).

MATERIALS AND METHODS

The chemicals purchased from indigenous companies were of pure and used for the analysis of various samples of our research.

Source of fertilized eggs and incubation conditions

Freshly laid *Bobcock strain* zero day old fertilized eggs were purchased from Sri Venkateswara Veterinary University, Tirupati and Sri Balaji hatcheries, Chittoor, Andhra Pradesh. The eggs were incubated horizontally at 37.5 ± 0.5ºC with a relative humidity of 65% in an egg incubator, we consider day1 (d1) as an incubation period of 24h. The humidity of the incubator was maintained by keeping the tray full of water inside. The water was replaced every alternate day and the water level was maintained to keep the same percentage of humidity throughout the incubation. Eggs were rotated manually four times a day and were examined through the Candler every day for the proper growth and viability. The dead eggs were removed immediately from the incubator. During all experiments, the embryos were maintained at 37 ± 0.5ºC except for brief intervals (60-120 seconds) required during the different treatment conditions. During this interval embryos experienced ambient room temperature (29-30ºC).

Treatment

Acrylamide treatment and collection of brain tissue

A group of six eggs (n = 6) were maintained for each time point and dose, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6mg, of acrylamide in saline was administered as single dose separately to fertilized chick embryos on day8 (d8), day9 (d9) and day10 (d10) of incubation. On 11th day the embryo's brain was collected and stored for further use at -20ºC.

Assay of Lipid peroxidation

Thiobarbituric acid reactive substance TBARS in tissues were estimated by the method of Fraga *et al*., 1988. The pink coloured chromogen formed by the reaction of 2-thiobarituric acid with breakdown products of lipid peroxidation was measured at 532nm.

Tissue processing for lipid peroxidation

Tissue homogenate10% of brain was prepared in 1.15% of KCl for lipid peroxidation. To 0.1mL of the tissue homogenate, added 0.2mL of 8.1% SDS and 1.5mL of 0.8% TBA. The total volume was made up to 4mL with distilled water and the tubes were kept at 95ºC for 60min, and then cooled. To this added 1ml of distilled water along with 5mL of n-butanol-pyridine mixture (15:1 v/v) and the contents were mixed vigorously. Then the tubes were centrifuged at 4000 rpm for 10 minutes and the color of the organic layer was measured at 532nm. A standard curve was plotted taking 1, 1, 3, 3-tetraethoxy propane as standard and the values of the samples were obtained from the standard curve.

Processing of tissue for antioxidant assay

Normal and treated chick embryo brain were thawed slowly, minced with scissors and homogenized in 50mM Tris-HCl buffer, pH 8.0, containing 0.25M sucrose and 1mM PMSF using a glass homogenizer. Homogenization was done by keeping the Potter Elvijhem homogenizer in an ice jacket and care was taken to minimize the froth formation. The homogenate was passed through two layers of cheese cloth to remove fat and the resulting supernatant was centrifuged at 35000 x g on high speed refrigerated centrifuge (Remi) for 30min. The resulted supernatant was used as the enzyme source. All the purification procedures were conducted at 4ºC unless otherwise stated.

Assay of non-enzymatic antioxidants

Reduced Glutathione (GSH)

Reduced Glutathione content was determined according to the method of Ellman and Boyne (Ellman *et al*., 1972). 1g of tissue was homogenized in10mL of phosphate buffer. From this 0.5mL was pipetted out and precipitated with 2.0mL of 5% TCA. 1.0mL of the supernatant was taken after centrifugation and added to it 0.5mL of Ellman's reagent followed by 3.0mL of phosphate buffer. The yellow colour developed was read at 412 nm. A series of standards were made in a similar manner along with a blank containing 3.5mL of buffer.

Ascorbic acid (Vit-C)

Ascorbic acid levels were determined according to the method of Omaye *et al*., 1979. 0.5mL of tissue homogenate was mixed thoroughly with 1.5mL of 6%TCA and centrifuged for 20 minutes at 3500 x g. To 0.5 mL of the supernatant, 0.5mL of DNPH reagent was added and mixed well. The tubes after 3h of incubation at room temperature placed in ice-cold water and added 2.5mL of 85% sulphuric acid and further allowed to stand for 30 minutes. A set of standards containing 10-

50mg of ascorbic acid were taken and processed similarly along with a blank, containing 0.5mL of 4% TCA. The color developed was read at 530 nm.

Assay of antioxidant enzymes

Catalase (CAT)

Catalase (EC.1.11.1.6) activity was measured by a slightly modified version of Aebi (1984), at room temperature. 10*i* L of absolute ethanol was added to 100*i* L of tissue extract as prepared above and then placed in an ice bath for 30 min. After 30min the tubes were kept at room temperature followed by the addition of 10*i* L of Triton X-100. The reaction mixture contained 200*i* L of phosphate buffer, 50*i* L of tissue extract and 250*i* L of 0.066M H_2O_2 in phosphate buffer. Decrease in optical density was measured at 240nm for 60sec in an Elico UV-VIS spectrophotometer. The molar extinction coefficient of 43.6 M cm^{-1} was used.

Glutathione Reductase (GR)

Glutathione reductase (EC.1.6.4.2) activity was determined by a slightly modified method of Carlberg and Mannervik (1985), at room temperature. The reaction mixture contained 50*i* L of 3mM NADPH in 0.1% $NaHCO_3$, 50*i* L of 20mM GSSG in phosphate buffer, 800*i* L of phosphate buffer and 100*i* L of enzyme source as prepared above. The reaction was monitored at 340nm for 3 min. The molar extinction coefficient of 6.22 x 10^3 M cm^{-1} was used to determine the GR activity. One unit of enzyme activity is equal to micromoles of NADPH oxidized/mg protein/min.

Glutathione Peroxidase (GPx)

Glutathione Peroxidase (EC.1.11.1.9) activity was assayed by the method of Rotruck *et al*. (1973). To 0.2mL of Tris buffer, 0.2mL of EDTA, 0.1mL of sodium azide and 0.5mL of homogenate were added. To the mixture, 0.2 mL of glutathione followed by 0.1mL of hydrogen peroxide was added. The contents were mixed well and incubated at 37ºC for 10 minutes along with a tube containing all the reagents except sample. After 10 minutes the reaction was arrested by the addition of 0.5 mL of 10% TCA, centrifuged and the supernatant was assayed.

Superoxide Dismutase (SOD)

Superoxide dismutase (EC.1.15.1.1) activity was determined according to the method of Misra and Fridovich (1972), at room temperature. The chick embryo brain tissue was homogenized in ice cold 50mM potassium phosphate buffer (pH7.0) containing 0.1mM EDTA to give 10% homogenate. The homogenate was centrifuged at 10,000rpm for 10min at 4ºC in cooling centrifuge. The resulting supernatant was used as the enzyme source for all the enzyme assays. 100ìL of enzyme was added to 880*i* L of 0.05M carbonate buffer, pH10.2, containing 0.1mMEDTA and 20*i* L of 30mM Epinephrine (in 0.05% acetic acid) was added to the mixture and measured the optical density values at 480nm for 4min in an Elico UV-VIS spectrophotometer. Activity expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to one unit.

Glutathione-S- Transferases (GSTs)

Glutathione-S- Transferases (EC.2.5.1.18) activity was determined according to the method of Habig *et al*. (1974). The reaction mixture contained 1.0mL of 100mM phosphate buffer, 0.1mL of 30mM CDNB, 0.1mL of homogenate and 0.7mL of distilled water. The reaction mixture was incubated at 37ºC for 5 minute then the reaction was started by the addition of 0.1mL of 30mM glutathione. The absorbance change was read at 340nm for 5 minutes. Reaction mixture without the enzyme was used as the blank.

Statistical analysis

Results were expressed as the means ± standard deviation (SD). Differences between groups were evaluated by using one-way ANOVA, followed by Dunett's t-test. All statistical analyses were performed using the statistical software SPSS 11.0 (SPSS Ltd., Surrey, UK). The p value of less than 0.05 was considered as statistically significant.

RESULTS

Effect of acrylamide on lipid peroxidation product

A statistically significant changes ($F = 17526.810$, $p < 0.05$) in MDA levels were observed in the brain of all six AC-treated 72h time of interval groups when compared to equal dose AC-treated 48 and 24h time of interval groups ($F = 8789.165$ and 8913.902, $p < 0.05$) (Fig. 1). In the 24h 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6mg AC groups, the MDA levels were 1.254, 1.556, 1.871, 2.226, 2.363 and 2.512 fold greater than in the control. Comparatively, increased lipid peroxidation in 48h, AC-treated group MDA levels, were 1.421, 1.632, 2.051, 2.375, 2.551 and 2.595 fold greater than in the control and 1.541, 1.763, 2.146, 2.549, 2.817 and 2.979 fold greater MDA levels were observed in 72h time of interval AC-treated groups than in the control group.

Effect of acrylamide on non enzymatic antioxidants

Glutathione (GSH)

The brain Reduced glutathione (GSH) levels of d(11) developing chick embryos control and AC-treated 24, 48 and 72h time of interval groups were presented in Fig. 2. The glutathione (GSH) levels at 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6mg AC-treated 24h time of interval groups were 5.716, 5.597, 4.583, 4.010, 3.666 and 2.978, respectively, and there was a 1.012, 1.034, 1.263, 1.443, 1.579 and 1.943 fold decrease was found in treated than in the control and 1.037, 1.120, 1.365, 1.474, 1.694, 2.014, 1.045, 1.205, 1.407, 1.579, 1.781 and 2.269 fold decrease in reduced glutathione levels were observed in the brain of all six AC-treated 48 and 72h time of interval groups when compared to control.

Ascorbic acid (Vit-C)

The brain ascorbic acid (Vit-C) levels of d(11) developing chick embryos control and AC-treated 24, 48 and 72h time of interval groups was presented in Fig. 3. The level of ascorbic acid (Vit-C) at 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6mg AC-treated 24h time of interval groups were 1.24, 1.09, 0.94, 0.85, 0.66 and 0.61, respectively and there was a 1.064, 1.211, 1.404, 1.552, 2.00 and 2.163 fold decrease in treated than in the control and 1.128, 1.257, 1.571, 1.692, 2.129, 2.490, 1.211, 1.419, 1.714, 1.783, 2.275 and 2.693 fold decreased ascorbic acid (Vit-C) levels were observed in the brain of all six AC-treated

Table 1: Effect of Acrylamide on LPO activity in Brain of 11th day old Chick Embryo

Treatment	24 h	48 h	72 h
Control	44.395 ± 0.45[a]	44.395 ± 0.45[a]	44.395 ± 0.45[a]
0.1 mg	55.705 ± 0.45[a]	63.115 ± 0.0.71[a]	68.443 ± 0.52[a]
0.2 mg	69.095 ± 0.38[b]	72.475 ± 0.38[b]	78.260 ± 0.47[b]
0.3 mg	83.070 ± 0.73[c]	91.065 ± 0.68[c]	95.290 ± 0.31[c]
0.4 mg	98.865 ± 0.21[d]	105.456 ± 0.42[d]	113.165 ± 0.45[d]
0.5 mg	104.910 ± 0.34[e]	113.295 ± 0.68[e]	125.060 ± 0.47[e]
0.6 mg	0.1476 ± 0.01[f]	115.245 ± 0.40[f]	132.275 ± 0.57[f]
ANOVA F-value	8913.902	8789.165	17526.810

Units: micromoles malondialdehyde formed per minute per milligram protein. Each value represents the mean ± SD (n = 6). Different letters are significantly different at the level of $p < 0.05$ according to the Scheffe's test

Table 2: Effect of Acrylamide on GSH activity in Brain of 11th day old Chick Embryo

Treatment	24 h	48 h	72 h
Control	5.789 ± 0.15[a]	5.789 ± 0.15[a]	5.789 ± 0.15[a]
0.1 mg	5.716 ± 0.16[e]	5.581 ± 0.13[f]	5.537 ± 0.08[f]
0.2 mg	5.597 ± 0.11[e]	5.166 ± 0.21[e]	4.801 ± 0.17[e]
0.3 mg	4.583 ± 0.15[d]	4.239 ± 0.21[d]	4.114 ± 0.20[d]
0.4 mg	4.010 ± 0.18[c]	3.927 ± 0.12[c]	3.666 ± 0.10[c]
0.5 mg	3.666 ± 0.19[b]	3.416 ± 0.15[b]	3.249 ± 0.20[b]
0.6 mg	2.978 ± 0.22[a]	2.874 ± 0.16[a]	2.551 ± 0.13[a]
ANOVA F-value	230.796	212.362	272.130

Units: mg/g tissue. Each value represents the mean ± SD (n = 6). Different letters are significantly different at the level of $p < 0.05$ according to the Scheffe's test

Table 3: Effect of Acrylamide on Vitamin-C activity in Brain of 11th day old Chick Embryo

Treatment	24 h	48 h	72 h
Control	1.32 ± 0.04[a]	1.32 ± 0.04[a]	1.32 ± 0.04[a]
0.1 mg	1.24 ± 0.04[e]	1.17 ± 0.04[e]	1.09 ± 0.04[e]
0.2 mg	1.09 ± 0.06[d]	1.05 ± 0.03[d]	0.93 ± 0.04[d]
0.3 mg	0.94 ± 0.06[c]	0.84 ± 0.04[c]	0.77 ± 0.04[c]
0.4 mg	0.85 ± 0.04[b]	0.78 ± 0.06[c]	0.74 ± 0.04[c]
0.5 mg	0.66 ± 0.04[a]	0.62 ± 0.03[b]	0.58 ± 0.04[b]
0.6 mg	0.61 ± 0.04[a]	0.53 ± 0.04[a]	0.49 ± 0.05[a]
ANOVA F-value	141.714	194.000	181.444

Units: mg/g tissue. Each value represents the mean ± SD (n = 6). Different letters are significantly different at the level of $p < 0.05$ according to the Scheffe's test

Table 4: Effect of Acrylamide on Catalase activity in Brain of 11th day old Chick Embryo

Treatment	24 h	48 h	72 h
Control	0.1624 ± 0.04[a]	0.1624 ± 0.04[a]	0.1624 ± 0.04[a]
0.1 mg	0.1985 ±0.05[a]	0.1912 ± 0.02[b]	0.1868 ± 0.01[a]
0.2 mg	0.1952 ± 0.05[a]	0.1716 ± 0.01[ab]	0.1638 ± 0.02[c]
0.3 mg	0.1742 ± 0.04[a]	0.1596 ± 0.01[a]	0.1542 ± 0.01[bc]
0.4 mg	0.1683 ± 0.06[a]	0.1575 ± 0.01[a]	0.1386 ± 0.01[abc]
0.5 mg	0.1597 ± 0.02[a]	0.1491 ± 0.01[a]	0.1301 ± 0.01[ab]
0.6 mg	0.1454 ± 0.03[a]	0.1400 ± 0.01[a]	0.1169 ± 0.03[a]
ANOVA F-value	1.108	5.715	11.449

Units: μmoles of H_2O_2 used per minute per mg protein. Each value represents the mean ± SD (n = 6). Different letters are significantly different at the level of $p < 0.05$ according to the Scheffe's test

48 and 72h time of interval groups and compared to control.

Effect of acrylamide on antioxidant enzymes

Catalase (CAT)

The effect of treatments with AC on the activity of catalase (CAT) in brain was shown in Fig. 4, respectively. A decrease in the activity of CAT of all six AC-treated brain tissue of developing chick embryos was noted in 24h ($F = 1.108$, $p < 0.05$), 48h ($F = 5.715$, $p < 0.05$) and 72h ($F = 11.449$, $p < 0.05$) (Fig. 4). The activity of CAT in the 24, 48 and 72h at the 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6AC dose group was statistically significantly ($p < 0.05$) decreased compared with the control group. Among the changes in CAT activity, the biggest decline (1.547 fold) was noted in 72h of the high dose AC (0.6 mg) group compared with controls.

Glutathione reductase (GR)

A parallel decrease in GR activity was noted in 24h ($F = 176.020$, $p < 0.05$), 48h ($F = 505.269$, $p < 0.05$) and 72h ($F = 457.218$, $p < 0.05$ (Fig. 5). Compared with controls, the activity of GR in the 24, 48 and 72h even at the low dose AC group showed a statistically significant ($p < 0.05$) decrease; but, the biggest declines were noted in 72h of the high dose AC (0.6 mg) group compared with controls (Fig. 5).

Glutathione peroxidase (GPx)

The effects of treatment with AC on the activity of glutathione peroxidase (GPx) in brain were shown in Fig. 6. A decrease in the activity of GPx of all six AC-treated brain tissue of developing chick embryos was noted in 24h ($F = 0.645$, $p < 0.05$), 48h ($F = 6.792$, $p < 0.05$) and 72h ($F = 7.618$, $p < 0.05$). The activity of GPx in the 24, 48 and 72h at the 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg AC dose group was statistically significantly ($p < 0.05$) decreased compared with the control group.

The Fig. 7 and 8 represents the levels of superoxide dismutase (SOD) and glutathione s-transferases (GSTs), respectively, in brain tissue of normal and all six AC-treated developing chick embryos. The embryos administered with acrylamide, showed a significant increase in the levels of SOD and GSTs activity as compared to control; the biggest activities were noted in 72h of the 0.4mg AC dose group when compared to controls and 0.5 and 0.6 mg AC-treated brain tissue of developing chick embryos at 48 and 72h time of interval were significantly decreased compared with the control group**.** Among the changes in SOD and GSTs activities, 1.703 and 1.401 fold biggest declines were noted in 72h of the high dose of AC (0.6mg) group compared with controls.

DISCUSSION

The recent discovery of AC at parts per million (ppm) levels in a wide variety of commonly consumed foods has energized research efforts worldwide to understand its adverse effects in different tissues in various experimental models (Hagmar *et al*., 2001; Rice, 2005; LoPachin *et al*., 2006; Manjanatha *et al*., 2006; Klaunig, 2008; Bowyer *et al*., 2009; Park *et al*., 2010; Wang *et al*., 2010). Although the genotoxic effects of AC and its reactive metabolite, GA, are well established in the liver of BB mice (Manjanatha *et al*., 2006), it is a multisite carcinogen, in mice, in studies examining only the lung and skin, acrylamide induced lung and/or skin tumors. In female rat's acrylamide induced tumors of the mammary gland, thyroid, central nervous system, oral cavity, uterus and clitoral gland and, in male rats, tumors of the thyroid, testis (mesothelioma), and central nervous system (Johnson *et al*., 1986; Friedman *et al*., 1995). Additionally, the central nervous

Table 5: Effect of Acrylamide on GR activity in Brain of 11th day old Chick Embryo

Treatment	24 h	48 h	72 h
Control	0.4557 ± 0.005^{a}	0.4557 ± 0.005^{a}	0.4557 ± 0.005^{a}
0.1 mg	0.4322 ± 0.007^{e}	0.4200 ± 0.007^{f}	0.3982 ± 0.007^{d}
0.2 mg	0.4133 ± 0.007^{d}	0.3998 ± 0.005^{e}	0.3850 ± 0.006^{d}
0.3 mg	0.3817 ± 0.004^{c}	0.3702 ± 0.003^{d}	0.3500 ± 0.006^{c}
0.4 mg	0.3738 ± 0.008^{bc}	0.3547 ± 0.005^{c}	0.3463 ± 0.004^{c}
0.5 mg	0.3655 ± 0.005^{b}	0.3292 ± 0.002^{b}	0.3012 ± 0.004^{b}
0.6 mg	0.3333 ± 0.003^{a}	0.3013 ± 0.005^{a}	0.2667 ± 0.005^{a}
ANOVA F-value	176.020	505.269	457.218

Units: nanomoles NADPH oxidised /minute/ milligram protein. Each value represents the mean ± SD (n = 6). Different letters are significantly different at the level of $p < 0.05$ according to the Scheffe's test

Table 6: Effect of Acrylamide on GPx activity in Brain of 11th day old Chick Embryo

Treatment	24 h	48 h	72 h
Control	0.1351 ± 0.03^{a}	0.1351 ± 0.03^{a}	0.1351 ± 0.03^{a}
0.1 mg	0.1785 ± 0.04^{a}	0.1871 ± 0.01^{b}	0.1910 ± 0.01^{b}
0.2 mg	0.1702 ± 0.03^{a}	0.1661 ± 0.01^{ab}	0.1548 ± 0.01^{a}
0.3 mg	0.1621 ± 0.02^{a}	0.1580 ± 0.01^{ab}	0.1531 ± 0.01^{a}
0.4 mg	0.1619 ± 0.03^{a}	0.1499 ± 0.01^{a}	0.1358 ± 0.01^{a}
0.5 mg	0.1573 ± 0.02^{a}	0.1380 ± 0.01^{a}	0.1318 ± 0.01^{a}
0.6 mg	0.1476 ± 0.01^{a}	0.1361 ± 0.02^{a}	0.1234 ± 0.03^{a}
ANOVA F-value	0.645	6.792	7.618

Units: μmoles of glutathione used per minute per g protein. Each value represents the mean ± SD (n = 6). Different letters are significantly different at the level of $p < 0.05$ according to the Scheffe's test

Table 7: Effect of Acrylamide on SOD activity in Brain of 11th day old Chick Embryo

Treatment	24 h	48 h	72 h
Control	15.29 ± 1.10^{a}	15.29 ± 1.10^{a}	15.29 ± 1.10^{a}
0.1 mg	15.39 ± 1.28^{a}	16.65 ± 2.4^{b}	17.30 ± 2.2^{b}
0.2 mg	17.77 ± 1.33^{ab}	18.32 ± 1.26^{bc}	21.08 ± 1.95^{c}
0.3 mg	20.39 ± 1.96^{bc}	20.67 ± 2.17^{c}	21.47 ± 1.97^{c}
0.4 mg	21.30 ± 1.85^{c}	22.07 ± 2.68^{c}	23.93 ± 2.3^{c}
0.5 mg	16.92 ± 1.23^{a}	14.90 ± 1.09^{ab}	12.35 ± 2.9^{a}
0.6 mg	16.87 ± 2.11^{a}	11.31 ± 3.5^{a}	8.97 ± 1.45^{a}
ANOVA F-value	11.087	16.669	41.606

Units: The amount of enzyme required to inhibit 50% NBT reduction/mg protein. Each value represents the mean ± SD (n = 6). Different letters are significantly different at the level of $p < 0.05$ according to the Scheffe's test

system is a target not only for acrylamide induced tumorigenicity, but also neurotoxicity. Human occupational studies and animal experiments have demonstrated acrylamide to be a potent neurotoxicant. The brain is particularly vulnerable to oxidative attack because it has a high rate of oxygen consumption, it contains a relatively poor level of antioxidants, and neuronal cell membranes are enriched in polyunsaturated lipids, which are readily attacked by free radicals, becoming oxidized into lipid peroxides (Floyd, 1999). Both *in vitro* and *in vivo* studies have shown that oxidative stress to neurons induced by reactive oxygen species (ROS) such as hydrogen peroxide, nitric oxide, superoxide and hydroxyl radicals, is presumed to be a key event in the pathology of AC and might contribute to amyloidogenesis and neuronal degeneration.

AC readily passes across blood–brain barrier into the CNS, where it is generated to free radicals. Three distinct pathways have been proposed: (1) AC conjugation with GSH, a metabolic process representing the major route of detoxification of AC. The process results in a rapid depletion of GSH and an overall decrease in cellular antioxidant contents; (2) liberation of glycidamide from AC metabolism. Glycidamide is a potent generator of ROS production (via inhibition of the mitochondrial respiratory chain) as well as an inhibitor of the activities of several antioxidant enzymes; (3) ROS generated as by-products of AC metabolism via cytochrome P450 2E1 oxidation. Reactive oxygen species (ROS) formed by lipid peroxidation are in fact required for cell functions if produced in physiological concentrations. Polyunsaturated fatty acids (PUFAs) synthesized from *ù* -3 and *ù* -6 essential fatty acids are key structural components of phospholipid membranes and are critical for brain growth, membrane fluidity, signal transduction, and visual and cognitive development. Normal brain function depends on maintaining homeostatic concentrations of PUFAs both during development and throughout life. However, impairments in PUFA metabolism have been implicated in many neurological diseases. Neuronal damage and death can also induce glial in activation and a localized, detrimental cycle of neuroinflammation by the release of toxic products by these cells, including reactive oxygen species (Griffin *et al*., 1998; Eikelenboom *et al*., 2002).

The data obtained in our laboratory confirm the statistically significant higher levels of MDA in brain of d (11) developing chick embryos after AC-treatment was observed (Fig. 1). AC-induced free radical production and LPO in neurons have been suggested to be responsible for the oxidative brain damage. This fact proves the intensification of lipid peroxidation in patients with central nervous system (Levchenko and Demchuk, 1991) as obtained similar results in patients operated on for a brain tumor glioblastoma multiforme and meningioma. The increase in lipid peroxidation concentration is proof of intensified oxidative free radicals (OFR) generation among other superoxide and hydroxyl radicals. An increase in oxidative free radicals (OFR) generation may be related to many biochemical reactions: Haber–Weiss or Fenton reaction, reaction catalyzed by xanthine oxidase, PUFA oxidation catalyzed by cyclooxygenase, or catecholamine oxidation, which leads to the formation of semiquinones and superoxide (Staub *et al*., 1994; Babior, 2002). In the opinion of the authors the growth of a tumour may be a cause of the disturbance of the equilibrium of redox

Table 8: Effect of Acrylamide on GST activity in Brain of 11th day old Chick Embryo

Treatment	24 h	48 h	72 h
Control	5.287 ± 0.93^{a}	5.287 ± 0.93^{a}	5.287 ± 0.93^{a}
0.1 mg	6.227 ± 0.29^{a}	6.727 ± 0.82^{a}	7.384 ± 0.36^{a}
0.2 mg	6.857 ± 0.46^{ab}	7.476 ± 0.84^{ab}	8.174 ± 0.36^{ab}
0.3 mg	7.491 ± 0.88^{bc}	8.336 ± 1.1^{ab}	8.725 ± 0.74^{bc}
0.4 mg	8.590 ± 1.0^{c}	9.105 ± 0.93^{b}	9.500 ± 0.98^{c}
0.5 mg	8.384 ± 1.0^{a}	8.321 ± 1.6^{ab}	8.189 ± 0.63^{ab}
0.6 mg	7.603 ± 0.2^{bc}	7.461 ± 0.38^{ab}	7.408 ± 0.63^{a}
ANOVA F-value	8.549	3.887	8.964

Units: μmoles of CDNB-GSH conjugates formed per minute per mg protein. Each value represents the mean ± SD (n = 6). Different letters are significantly different at the level of $p < 0.05$ according to the Scheffe's test

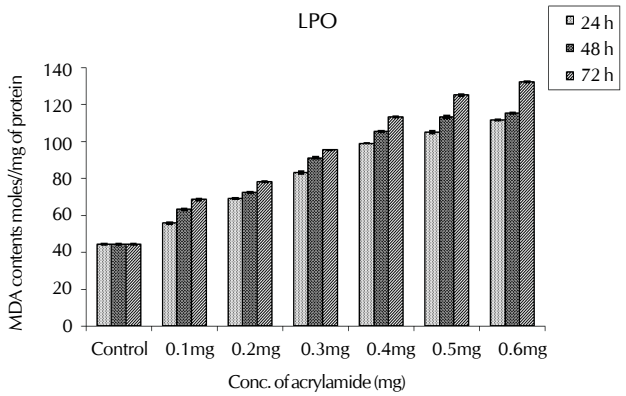

Figure 1: Effect of acrylamide on the lipid peroxidation of d (11) developing chick embryo brain. Brain of control, acrylamide of increasing quantities of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg per100 μL respectively, were assessed for a period of 24, 48 and 72h. The LPO level is expressed as micromoles malondialdehyde formed per minute per milligram protein.

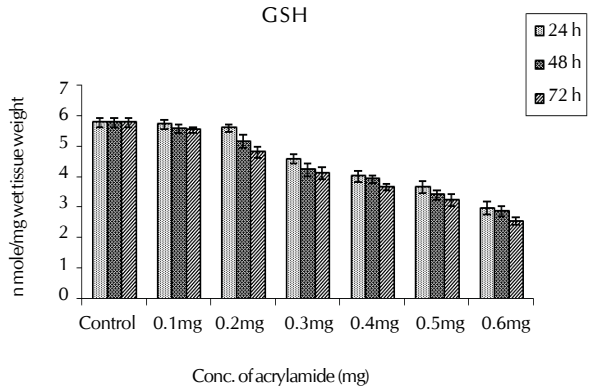

Figure 2: Effect of acrylamide on the glutathione level of d (11) developing chick embryo brain. Brain of control, acrylamide of increasing quantities of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg per100 μL respectively, were assessed for a period of 24, 48 and 72h. The glutathione level is expressed as mg/g tissue

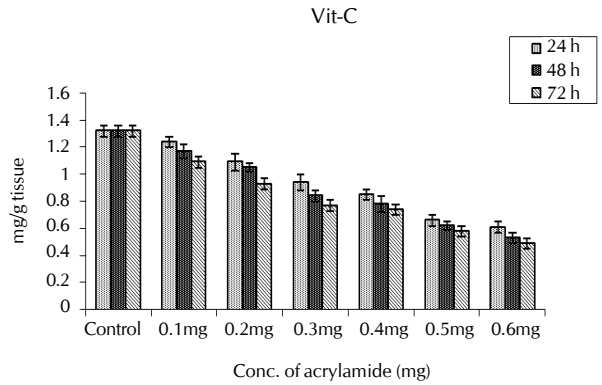

Figure 3: Effect of acrylamide on the ascorbic acid level of d (11) developing chick embryo brain. Brain of control, acrylamide of increasing quantities of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6mg per100 μL, respectively, were assessed for a period of 24, 48 and 72h. The ascorbic acid was expressed as mg/g tissue

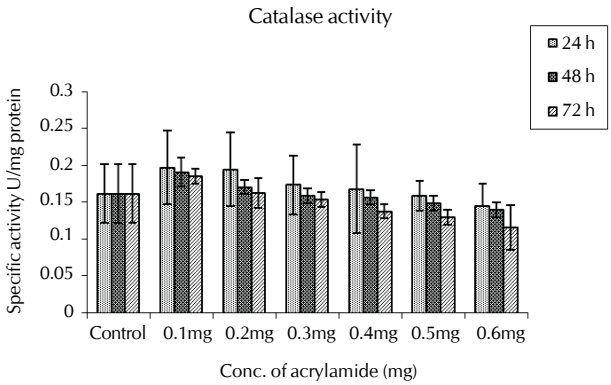

Figure 4: Effect of acrylamide on the catalase activity of d (11) developing chick embryo brain. Brain of control, acrylamide of increasing quantities of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg per100μL respectively, were assessed for a period of 24, 48 and 72h. The enzyme activity is expressed as micromoles H_2O_2 consumed per minute and milligram of protein

reactions with lipid peroxidation intensification. Louw *et al.,* 1997 showed an increase in the lipid peroxidation process in patients with astrocytoma. According to them intensified peroxidation may be responsible for the enlarged quantity of peroxisomes in tumour tissue. Some reports (Landolt *et al.,* 1994; Arora *et al.,* 1996) indicate that a higher level of oxygen-derived free radicals may be responsible for the intensification of the proliferation of neoplasmatic cells, which is followed by the development of a tumour. Arora *et al*., 1996) showed that the addition of a well-known free radical scavenger as lazaroids to brain tumour cell cultures leads to inhibition of neoplasm growth. Landolt *et al*. (1994) instead proved a decrease in the concentration of free radical scavengers (ascorbic acid, reduced glutathione and cysteine) in cells of astrocytoma which suggests an increase in oxidative free radicals (OFR).

Our results of developing chick embryo upon treatment with AC showed decrease in GSH and Vit-C levels (Fig. 2 and 3) indicated that the free radical removal capacity reduction in neurons system of chick embryo.

Mitochondrial respiration is the main biological source of superoxide anion radicals in physiological conditions. SOD is considered the first line of defense against deleterious effects of oxyradicals in the cell by catalyzing the dismutation of superoxide radicals to H_2O_2 and molecular oxygen. Also our results showed decrease in the activities of catalase (CAT) (Fig. 4), glutathione reductase (GR) (Fig. 5), glutathione peroxidase (GPx) (Fig. 6) and superoxide dismutase (SOD) (Fig. 7). A reduction in the activity of SOD causes a rise in the level of superoxide anion, which is known to inactivate catalase activity (Kono and Fridovich, 1982). Catalase or GPx fails to eliminate H_2O_2 from the cell and the accumulated H_2O_2 has been shown to cause inactivation of SOD (Sinet *et al*., 1981), and in addition to their direct effect on the cellular constituent, to produce oxidative stress by decreasing the enzymatic defenses. Therefore the AC molecule in the brain causes elevation of $O_2^{\cdot}$, H_2O_2 and decrease of Vit-C and GSH. Because of this result the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) were further reduced as

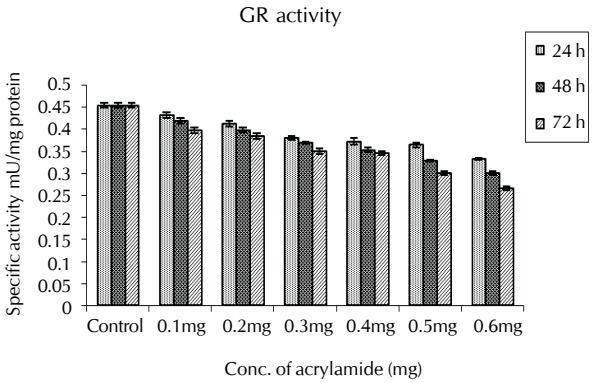

Figure 5: Effect of acrylamide on the glutathione reductase activity of d (11) developing chick embryo brain. Brain of control, acrylamide of increasing quantities of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg per100 μL respectively, were assessed for a period of 24, 48 and 72h. The enzyme activity is expressed as nanomoles NADPH oxidized /minute/ milligram protein

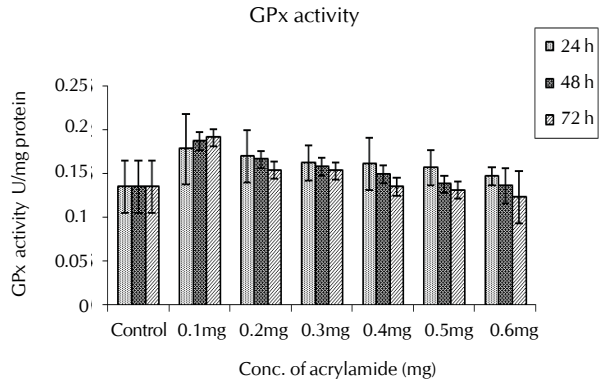

Figure 6: Effect of acrylamide on the glutathione peroxidase activity of d (11) developing chick embryo brain. Brain of control, acrylamide of increasing quantities of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg per100 μL respectively, were assessed for a period of 24, 48 and 72h. The enzyme activity is expressed as nanomoles NADPH oxidized /minute/ milligram protein.

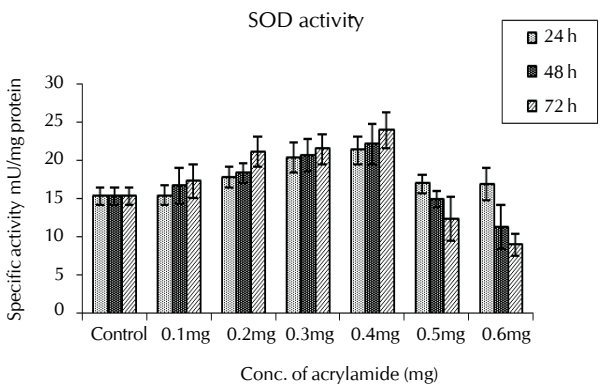

Figure 7: Effect of acrylamide on the superoxide dismutase activity of d (11) developing chick embryo brain. Brain of control, acrylamide of increasing quantities of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg per100 μL respectively, were assessed for a period of 24, 48 and 72h. The enzyme activity is expressed as the amount of enzyme required to inhibit 50% NBT reduction/mg protein

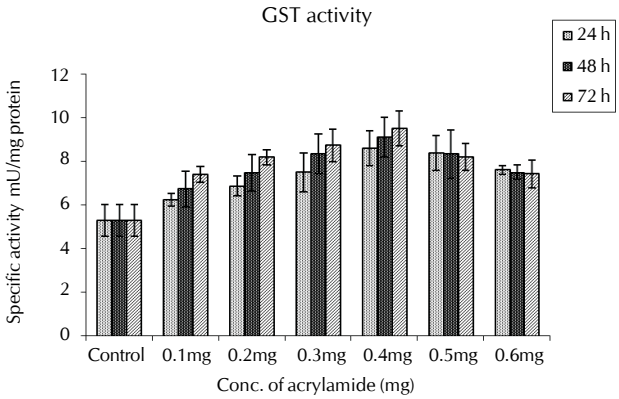

Figure 8: Effect of acrylamide on the glutathione s-transferases activity of d (11) developing chick embryo brain. Brain of control, acrylamide of increasing quantities of 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg per100 μL respectively, were assessed for a period of 24, 48 and 72h. The enzyme activity is expressed as μmoles of CDNB-GSH conjugate formed per minute per mg protein

indicated in Fig. 4, 5 and 6. Actually the antioxidant enzymes catalase and peroxidase protect SOD against inactivation by H_2O_2. Reciprocally, the SOD protects catalase and peroxidase against inhibition by superoxide anion. This mutual protection process was observed to be eliminated in developing chick embryo brain by acrylamide. At low H_2O_2 generation rates, GPx plays a key role in H_2O_2 metabolism, while at higher H_2O_2 generation rates; the role of CAT becomes more important. Thus, balance of this enzyme system may be essential to eliminate superoxide anion and peroxides generated in brain. GPx, a selenium containing antioxidant enzyme, removes peroxyl radicals from various peroxides including H_2O_2, whereas GR regenerates reduced glutathione from its oxidized form (Sikka, 2001).The reduction in catalase activity reflects the inability of brain to eliminate H_2O_2 produced or to enzyme inactivation caused by excess ROS production in brain (Pigeolet *et al*., 1990). In the present study, the reduction in the activities of SOD, catalase, GPx and GR and enhanced in the levels of LPO and H_2O_2 could reflect the adverse effect of acrylamide on the antioxidant enzymes in brain tissue of developing chick embryos.

In addition to above in elimination of stress based on oxygenated molecules and acrylamide alone the conjugation of AC with GSH catalyzed by GST to form mercapturic acid was considered as a major pathway for AC metabolism (Dixit *et al*., 1981) in chick embryos. However, Dixit *et al*. (1981) and Ruxana and Thyaga Raju (2010) have suggested that AC inhibits hepatic GST, which may result in increased metabolism of AC to GA via the cytochrome P-450 pathway. In the present study, examination of total GST activity in the AC- treated chick embryo showed that total GST activity was significantly higher up to 0.4mg treatment following decrease from 0.5-0.6 AC exposure, compared to controls (Fig. 8) as reported earlier by Vasundara *et al.,* 2006 in rats**.** Higher GST activity was also associated with a significant decrease in GSH levels (Fig. 2) in AA-treated chick embryos, suggesting an

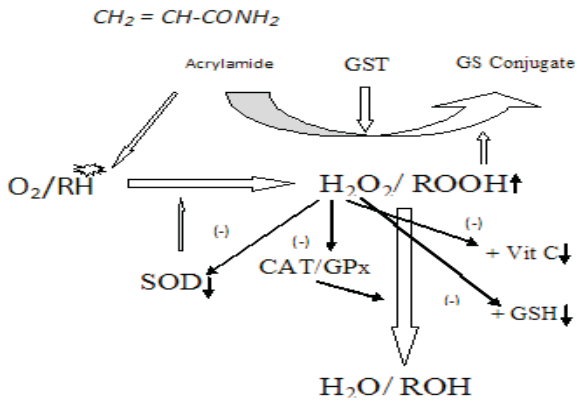

Figure 9: Proposed mechanism of acrylamide influence on oxygenated molecules and their antioxidants in chick embryo brain based on the results obtained.

enhanced rate of conjugation of AA with GSH. An AA-mediated concentration related increase in GST activity and a decline in GSH levels were also found in isolated brain tissues. In the present study, a significant fall in GSH levels was also evident in GA-treated chick embryos despite the fact that there was no significant change in GST activity.

CONCLUSION

In conclusion from our study (Table 1 to 8) it was observed that the AC can reduce antioxidant contents and enzymes. In chick embryo brain tissue because of reduction of GSH and Vitamin C the free radical scavenger's role was suppressed by AC and the enzymes that can eradicate the function of AC and its products were induced up to 0.4mg treatment and later reduced as studied by the earlier scientists on hepatic tissues of chick embryos, mice and rat. The suppression of all antioxidants function by AC may suggest that it is probably involved in the propagation of toxicity to central nervous system by damaging the neurons that are involved in the nerve impulse transmission.

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