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Rat Brain Glutathione S-transferasespotentially Defends Acrylamide Induced Neurotoxicity and Genotoxicity

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Abstract: *Acrylamide (AC) is a noxious chemical and it is widely used in this modernized world. There by this study aimedto assess the acrylamide induced neurotoxicity and genotoxicityonmale wistar rat brain. Based on the study aim, male at brainwas exposed by short term and long term to acrylamide and investigated the crucial changes in glutathione S-transferases and its associated glutathione peroxidase activities including degradation of DNA. Significant alterations of glutathione S-transferases and its associated glutathione peroxidase activities andinjured DNA results were documented. This study revealed that the glutathione S-transferases are potentially defends acrylamide toxicity to some extent but their activities aresignificantly decreasedby the accumulation of acrylamidein the brain and leads to degradation of DNA.*

Keywords: *acrylamide, glutathione S-transferase, glutahtione peroxidase and genotoxicity*

I. Introduction

Now a days acrylamide (AC) is widely used in variouscommercial products [1, 2]. That the AC induced neurotoxicity was characterized by ataxia, skeletal muscle weakness, cognitive impairment and numbness in the occupational exposure [3,4,1]. The majority studies said that the AC target thenervous tissue and studies on rodents also suggested that AC causes reproductive toxicity thereby decreased litter size and genotoxicity such as DNA strand breaks [5].That the nerve terminals are primary site of AC action, as a result impaired synaptic transmission has been impaired in both peripheral nervous system (PNS) and central nervous system (CNS) [6,7].Acrylamide causes genotoxicity by binding its metabolite (glycidamide) with DNA [8] and itrelease the large amount of free radicals in to body there by ACgenerate disturbances between the oxidative status and antioxidant enzymatic system [9]. In tobacco smoke high levels of acrylamide (AC) wasidentified [10].Several studies have been concluded the interaction of AC with GST in both rats and mice [11, 12]. Generally AC is formed during cooking foods at high temperatures that is more than 120^{o}C.

Glutathione S-transferases (GST) EC 2.5.1.18,are a multifunctional dimeric protein involved in cellular detoxification of reactive electrophilic compounds, and protecting tissues against oxidative damage [13]. Generally GSTs catalyse the conjugation reactions but certain class of GST can also catalyse the reduction of cellular peroxides like fatty acid hydroperoxides[14]. This kind of peroxidase activity is associated with the GSTs which is a selenium independent activity and it is an important feature that existin cells can protect against hydroperoxides known as reactive oxygen species (ROS) [14].Increasing the expression level of the GPx activitycan protect cells from free radicals [15]. This study was aimed to investigate the effect of AC on rat brain GSTs and GST associated GPxactivities and degradation of DNA.

II. Materials and Methods

1.1. Chemicals

Acrylamide (AC) (99.9%), glutathione (GSH), 1-chloro 2,4-dinitrobenzene (CDNB), ethylenediaminetetraaceticacid(EDTA), agarose, Cumene hydroperoxide(CHP), NADPH, Glutathione reductase(GR) were purchased from Merk chemicals, USA. Sucrose, Hydrochloric acid (HCL), Hydroxymethylaminomethane (Tris base),glycine,glycerol, sodium carbonate, sodium thiosulphate,boric acid, formaldehyde, phenylmethanesulphonylfluoride(PMSF), sodium dodecylsulphate (SDS),bromo phenol blue, ethidiumbromide and other chemicalsprocuredfromthehimediachemical company, Mubai, India.

1.2. Methodology

1.2.1. Maintenance of animals

The experiments wereconducted on 18 malerats weighing about 150 to 200grams. Animals were acclimatized for about one week and housed in plastic cages. During the experimental period, they were housed under standard laboratory conditions, 12:12 light/dark photoperiod at 23 ± 2 ºC. The animals fed with ad libitum and water throughout the study.

1.2.2. Experimental design

Short term treatment: Male wistar rats (n=6) weighing about 150 to 200 grams were injected intraperitoneally with 2 mg of acrylamide, distilled water as vehicle for 24 hours per each injection for six doses to a total of 12 mg. Long term treatment: Male wistar rats (n=6) weighing about 150 to 200 grams were injected intraperitoneally with 2 mg of acrylamide, distilled water as vehicle for 72 hours per each injection for six doses to a total of 12 mg.Control animals (n=6) received vehicle only

1.2.3. Tissue collection and sample preparation

Both control and treated rat were weighed and decapitated under anesthesia and separated brain was washed with 50 Mm Tris HCL buffer (pH 8.0), containing 1 mMehylenediaminetetraacetic acid (EDTA) in order to remove excess blood and body fluids, instantly collected tissues were preserved at -20^{o}C for GST and GPx activity assays and genotoxicity study.At the time of experimentation the collected normal and treated rat brain tissues were slightly thawed and 20% of brain tissue homogenate was prepared in cold 50 mMTris HCL buffer, (pH 8.0), containing 0.25M sucrose and 1mM phenylmethanesulphonyl fluoride (PMSF) using a potter Elvijhem 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 floating lipid materials and the resulting supernatant was centrifuged at 10,000 rpm by using refrigerated centrifuge two times for 45 min at 4^{o}C. The collected supernatant was known as cytosolic fraction and it was used as the enzyme source for activity assays.

1.2.4. Glutathione-S-transferase (GST) activity assay

GST catalyses the conjugation reaction with glutathione in the first step of mercapturic acid synthesis and GST activity was measured by the method [16]. The reaction mixture contained suitable amount of the enzyme (25 µg of protein in homogenate), 1 ml of KH_2PO_4buffer, 0.2 ml of EDTA, 0.1 ml of 1-chloro-2,4-dinitrobenzene (CDNB), and GSH. The reaction was carried out at 37^{o}C and monitored spectrophotometrically by the increase in absorbance of the conjugate of GSH and CDNB at 340 nm. A blank was run without enzyme. One unit of GST activity is 1 µmol product formation per minute.

1.2.5. Glutathione peroxidase (GPx) activity assay

Glutathione peroxidase (GPx) activity was measured by NADPH oxidation, using a coupled reaction system consisting of glutathione(GSH), glutathione reductase(GR), and cumenehydroperoxide(CHP) [17]and the reaction is follow, 100 µL of enzyme sample was incubated for five minutes with 1.55 ml stock solution (prepared in 50 mMTris HCL buffer (pH 7.6) with 0.1 mM EDTA) containing 0.25 mM GSH, 0.12 mM NADPH and 1 unit glutathione reductase(GR). The reactionwas initiated by adding 50 µLof cumenehydroperoxide (CHP)(1mg/ml), and the rate of disappearance of NADPH with time was determined by monitoring absorbance at 340 nm. One unit of enzyme activity is defined as the amount of enzyme that transforms 1 µmol of NADPH to NADP per minute.

1. 2. 6.Genotoxicitysudies

Both control and treated brain tissue slices were placed in the digestion buffer (50 mMTris HCLwith pH 8.0; 0.1 M EDTA with pH 8.8; 1% SDS and proteinase K with concentration of 1 mg/10 ml) and allowed for overnight incubation at 55^{o}C in water bath and DNAwas extracted from digestive samples by the conventional method of 10% saturated phenol/chloroform/isoamyl alcohol (24:24:1) and DNA was precipitated by using ice cold ethanol. The precipitated DNAwas rinsed two times with 70% ethanol and DNA was allowed for air dry and DNA was dissolved in Tris EDTA buffer. DNA quantification measured by the method [18]using a spectrophotometer at 260nm/A280nm and only samples with 1.8 ratios were used for further experimentation. To analyse the DNA fragmentation, electrophoresis was carried out by 1%agarose gel containing ethidium bromide. The gel was examined under UV light and visualized DNA bandswere photographed.

III. Results

1.3. Activity of Glutathione S-transferasesand Glutathione peroxidase

	CDNB	CHP
Control	7.29^{a} ± 0.09	7.09^{a} ± 0.23
2 mg	9.51^{b}± 0.32	9.32^{b}± 0.50
4 mg	11.2^{b} ± 0.06	10.89^{b} ±0.62
6 mg	8.01^{b} ± 0.10	7.93^{b} ± 0.41
8 mg	5.11^{b} ± 0.21	5.03^{b} ± 0.04
10 mg	2.06^{b} ± 0.04	1.98^{b} ± 0.33
12 mg	0.9^{b} ± 0.03	0.8^{b} ± 0.02

Table-1: Influence of short term treated acrylamide (AC) on the levels of rat brain GSTs and GPx

One unit of enzyme activity is defined as micromoles of GSH conjugate formed/min/mg protein (CDNB). One unit is defined as micromoles of NADPH oxidized/min/mg protein (CHP).
Values are average of three separate experiments of three samples. Mean ±SD significant (t-test). a = $p<0.01$ b = $p<0.05$

As shown in fig. 1, in short term exposure to acrylamide, GST activity was increased by 63% and 74% in response to 2 mg and 4 mg but gradually decreasedby 53%, 34%,13% and 6% in response to 6 mg, 8 mg, 10 mg and 12 mg when compared to control i.e 60.7%. Glutathione peroxidase (Selenium Independent) activity was increased by 62% and 72% in response to 2 mg and 4 mg but gradually decreased by 52%, 33%, 13% and 5% in response to 6 mg, 8 mg, 10 mg and 12 mg when compared to control i.e 59%. As shown Table.1, significant changes were observed in both GST and GPx activities (a = $p<0.01$ and b = $p<0.05$).

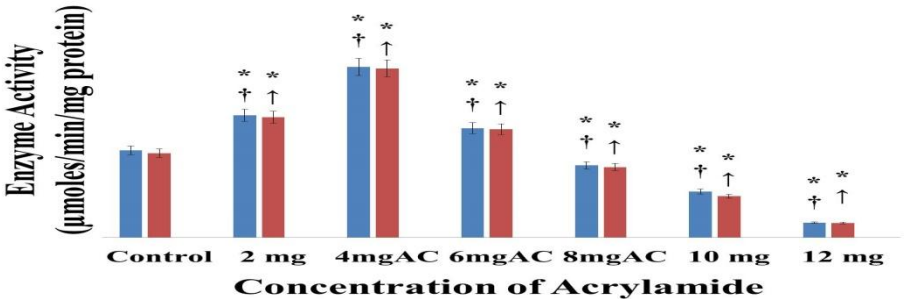

Figure 1: Acrylamide induced alterations in brain GST andGPxactivities in short term treatment

In this figure, GST activity represented as †, GST associated GPx activity represented as ↑; significance as *

As shown in fig. 2, in long term exposure to acrylamide, GST activity was increased by 43% and 60% in response to 2 mg and 4 mg but gradually decreased by 38%, 25%, 16% and 5% in response to 6 mg, 8 mg, 10 mg and 12 mg. Glutathione peroxidase (Selenium Independent) activity was increased by 42% and 59% in response to 2 mg and 4 mg but gradually decreased by 38%, 24%, 14% and 5% in response to 6 mg, 8 mg, 10 mg and 12 mg. As shown Table.2, significant changes were observed in both GST and GPx activities (a = $p<0.01$ and b = $p<0.05$).

	CDNB	CHP
Control	$6.14^{a} \pm 0.31$	$5.95^{a} \pm 0.20$
2 mg	$8.62^{b} \pm 0.40$	$8.48^{b} \pm 0.62$
4mgAC	$12.03^{b} \pm 0.5$	$11.92^{b} \pm 0.5$
6mgAC	$7.71^{b} \pm 0.21$	$7.63^{b} \pm 0.51$
8mgAC	$5.09^{b} \pm 0.41$	$4.96^{b} \pm 0.4$
10 mg	$3.24^{b} \pm 0.10$	$2.91^{b} \pm 0.20$
12 mg	$1.05^{b} \pm 0.21$	$1.02^{b} \pm 0.10$

Table-2: Influence of long term treated acrylamide (AC) on the levels of rat brain GSTs and GPx
One unit of enzyme activity is defined as micromoles of GSH conjugate formed/min/mg protein (CDNB). One unit is defined as micromoles of NADPH oxidized/min/mg protein (CHP).
Values are average of three separate experiments of three samples. Mean ±SD significant (t-test). a = $p<0.01$ b = $p<0.05$

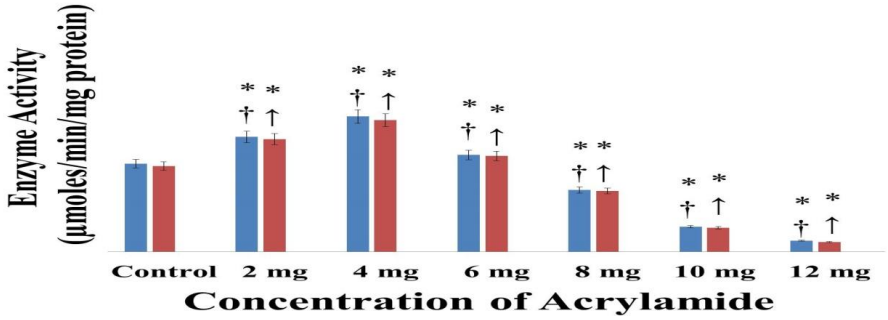

Figure 2: Acrylamide induced alterations in brain GST and GPx activities
In this figure, GST activity represented as †, GST associated GPx activity represented as ↑, significance as *

In this study, gradual increase and gradual decrease can be seen in rat brain GST activity and GST associated GPx activityin both short term and long term exposure to acrylamide. GST transferaseactivity was increased linearly when acrylamide infiltrated into brain, at that point peroxidase activity associated with GST was increased and GST transferase activity was decreased linearly when acrylamide concentration was increased in brain, at that point peroxidase activity associated with GST was decreased. This is because; primarilyGSTs and GPx activities were increased linearly to remove low quantity of acrylamide. Finally, high quantity accumulation of acrylamide destabilizes the activity of GST and GPx and there by GST and GPx activities were decreased linearly.

1.4. Genotoxicityevaluation

Rat brain DNA fragmentation was evaluated by agarose gel electrophoresis. DNA was degraded at the concentration of 12 mg in both short term and long term treatment of acrylamide. Degraded DNA was visualized as a longstreak when compared to control under UV light as shown in Fig. 3. But 12 mg acrylamide damaged more in long term exposure than the short term exposure of the same concentration.

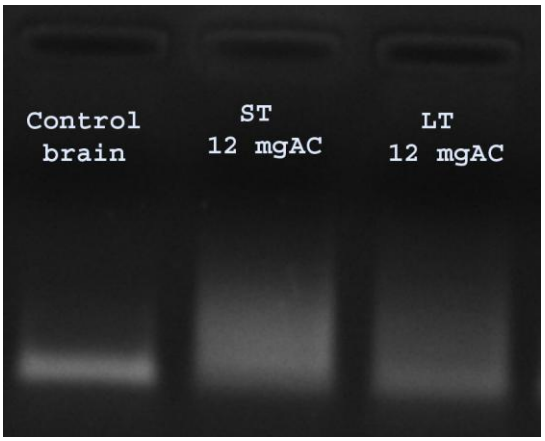

Figure: 3. 1% agarose gel electrophoresis showing degradation of DNA by acrylamide.

In this figure: Control brain DNA, short term (ST) 12 mg AC and long term (LT) 12 mg AC

IV. Statistical analysis

All the data related to this study and their results were calculated from three experiments and presented as the mean ± standard deviation (SD). Student t-test was performed to identify the acrylamide treated rat brain samples differed from the mean for the respective vehicle controls. That the differences between the experimental groups at the level of $P < 0.05$ were considered as significantwhen compared to control $p<0.01$.

V. Discussion

Biological cells handle the ROS by using glutathione antioxidant system which includes glutathione S-transferases (GSTs), glutathione peroxidase (GPx) and glutathione reductase (GR), this enzymatic system is protect cells from oxidative stress [19, 20].Acrylamide (AC) is a carbohydrate rich food contaminant in addition to environmental contaminant.AC is an electrophile and it causes cytotoxicity by interaction with nucleophile residues of biological macromolecules such as enzymes and DNA through covalent bonds [21, 22, 23]. By oral administration AC can rapidly absorbed into the circulation, there by distributed to vital organs and reacts with hemoglobin, nerve cells and enzymes including DNA[24]. AC can suppress metabolism and axonal transport in neurons which leads to deficiency of nutritional factors [25, 26]. Acrylamide shows marked efficiency binding to brain [27]. In both laboratory animals and humans, neurotoxicity of AC is characterized by ataxia and distal skeletal muscle weakness [28].

GSTs are key enzymes to defend cell against cytotoxic and carcinogenic agents [29]. Life span and memory performance is reduced by low level expression of GST in ~~ANA~~ rats [30].GSTs catalyse the conjugation of GSH with CDNB [31]. GSTs associated peroxidase activity is referred to as Non-Selenium GPx activity and it protect the cells from hydroperoxides also known as reactive oxygen species (ROS) [14].Selenium independent glutathione peroxidase activity is associated with GSTs [32].

As in the result part i.e 3.1, inboth short term and long term exposure to acrylamide, GST activity was increased with the substrate CDNB by 63% and 74% in response to 2 mg AC and 4 mg AC but gradually decreased by 53%, 34%, 13% and 6% in response to 6 mg AC, 8 mg AC, 10 mg AC and 12 mg AC and 43% and 60% in response to 2 mg AC and 4 mg AC but gradually decreased by 38%, 25%, 16% and 5% in response

to 6 mg AC, 8 mg AC, 10 mg AC and 12 mg AC when compared to controli.e 60%. In both short term and long term exposure to acrylamide, glutathione peroxidase (Selenium Independent) activity was increased with the substrate CHP by 62% and 72% in response to 2 mg AC and 4 mg AC but gradually decreased by 52%, 33%, 13% and 5% in response to 6 mg AC, 8 mg AC, 10 mg AC and 12 mgACand by 42% and 59% in response to 2 mg AC and 4 mg AC but gradually decreased by 38%, 24%, 14% and 5% in response to 6 mg AC , 8 mg AC, 10 mg AC and 12 mg compared to control i.e59%. The present study was agreed with [31, 14, 32].

Due to oxidation of the double bond between α and β carbons, that the acrylamide is metabolised into a reactive epoxide that isglycidamidein liver [33] and this epoxide induces strand breakage by the production of DNA adducts [34]. Acrylamide induce DNA damage and generates oxidative changes in rat brain [35, 36].Acrylamide genotoxicity is promoted both chromosomal clastogenesis and DNA-strand breakage [37].

As in result part i.e 3.2, inboth short term and long term exposure to acrylamide, DNA was degraded and damaged DNA was visualized ~~a smear~~as a long streak when compared to control, as shown in fig. 3. The present study was agreed with [35, 36, 37].

VI. Conclusion

Both short term and long term exposure of rats to acrylamide was evidenced by significant alterations in brain GST and GST associated GPx activities and degradation of DNA. This study showsthat the acrylamide induces significantchanges in both GST and GST associated GPx activities at enzymatic level as well as adversealterations in DNA at the level ofgenetic material. Key findings of this study, that the conjugation activity and reduction activity of GSTs towards a wide variety of electrophilic compounds, reveals that the dual activities are an integral part of the cellular defence system.So GST have two activities, with transferase activity GST catalyse conjugation ofGSH with acrylamide and with peroxidase activity GST catalyse reduction of GSH with lipid hydroperoxides generated by acrylamide. BothGST and GSTperoxidase activities are destabilized by the excess accumulation of acrylamideand itleads to interaction with brain DNA. Due to this interaction DNA was damaged. The present studyresult may contribute to understand the acrylamide induced neurotoxicity and genotoxicity and it requires further studies.

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