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Acrylamide Induced Alterations In Glutathione S-Transferase And Glutathione Peroxidase Activity And DNA Damage In Rat Brain

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Abstract: ***Acrylamide (AC) is a noxious chemical and it is widely used in this modernized world. There by this study aims to assess the harmful effects on male wistar rat brain. Based on the study aim, male rat brain was exposed by short term and long term to acrylamide and investigated the crucial changes in glutathione S-transferases (GST) and selenium dependent and selenium independent glutathione peroxidase (GPx) activities and the results were documented and AC was badly change in GST and GPx activities (P <0.05) and badly injured DNA. This study revealed that the acrylamide can induce significant changes in both antioxidant enzyme activities (GST and GPx) and DNA.***

Keywords: Acrylamide (AC), Glutathione S-transferase (GST), Glutahtione peroxidase (GPx) and DNA damage.

I. INTRODUCTION

An imbalance between pro-oxidants and antioxidants occurs in cells due to oxidative stress (Nazıroğlu, 2007). Oxidative stress leads to atherosclerosis, inflammation, ischemia-reperfusion injury, gene mutation, carcinogenesis, and tissue injury (Halliwell, 2014). Among the antioxidant enzymes glutathione S-transferase (GST) and Glutathione Peroxidase (GPx) are prominent. 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 (Hayes et al., 2005). Glutathione peroxidase (GPx) has various forms, containing or not containing selenium (Nakane et al., 1998). GPx capture reactive oxygen species (ROS) and thereby it protects the cells against oxidative stress. Increasing the expression level of the GPx enzyme can protect cells from free radicals (Koral Taşçi and Gülmez, 2014).

Acrylamide (AC) is a noxious chemical with formula of CH_2=CH–CO–NH_2 and it is a highly reactive small organic molecule with hydrophilic nature. Acrylamide can rapidly absorb and distribute throughout the body because of its small size, solubility in water and capability of reaction (Mannaa *et al.,* 2006). It is a well proved toxic chemical including neurotoxicity, reproductive toxicity, carcinogenicity, genotoxicity and mutagenicity (Chen et al., 2013). Acrylamide causes genotoxicity by binding to its metabolite (glycidamide) with DNA (Belinda et al., 2012) and It release the large amount of free radicals in to body there by it generate disturbances between the oxidative status and antioxidant enzymatic system (Yousef & El-Demerdash, 2006). In tobacco smoke high levels of acrylamide (AC) were identified (Pruser and Flynn, 2011). Several studies have been concluded the interaction of AC with GST in both rats and mice (Alturfan *et al.,* 2011; Rawi *et al.,* 2012). Generally AC is formed during cooking foods at high temperatures that is more than 120^{o}C. This study aimed to investigate the effects of AC on rat brain and AC was administered as short term and long term.

II. MATERIALS AND METHODS

Acrylamide (99.9%), Glutathione, (GSH), 1-chloro 2,4-dinitro benzene(CDNB), Ethylenediaminetetraacetic acid (EDTA), Agarose, Cumene Hydroperoxide (CHP), Hydrogen Peroxide (H_2O_2), NADPH, Glutathione reductase (GR) were purchased from Merk chemicals, USA. Sucrose, Hydrochloric acid (HCL), Hydroxymethyl aminomethane (Tris), glycine, glycerol, sodium carbonate, formaldehyde, phenylmethanesulphonyl fluoride (PMSF),

Sodium dodecyl sulphate (SDS), Ethidium bromide and other chemicals procured from the himedia chemical company, Mubai.

MAINTENANCE OF ANIMALS

The experiment was conducted on 18 male rats weighing 150-200 grams. 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.

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

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 mM ehylenediaminetetraacetic 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 DNA damage analysis.

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 50 mM Tris-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.

GLUTATHIONE-S-TRANSFERASE (GST) ACTIVITY: GST catalyses the conjugation reaction with glutathione in the first step of mercapturic acid synthesis and GST activity was measured by the method of Habig et al (1974). The reaction mixture contained suitable amount of the enzyme (25 µg of protein in homogenates), 1 ml of KH2PO4 buffer, 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 in absence of the enzyme. One unit of GST activity is 1 µmol product formation per minute.

GLUTATHIONE PEROXIDASE (GPX) ACTIVITY: Glutathione peroxidase (GPx) activity was measured by NADPH oxidation, using a coupled reaction system consisting of glutathione, glutathione reductase, and cumene hydroperoxide (Lawrence and Burk, 1976). 100 µL of enzyme sample was incubated for five minutes with 1.55 ml stock solution (prepared in 50 mM Tris buffer, pH 7.6 with 0.1 mM EDTA) containing 0.25 mM GSH, 0.12 mM NADPH and 1 unit glutathione reductase. The reaction was initiated by adding 50 µL of cumene hydroperoxide (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 and results are expressed as units/mg protein.

DNA ISOLATION AND FRAGMENTATION STUDIES

Both control and treated brain tissue slices were placed in the digestion buffer (50 mM Tris HCl with 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 DNA was 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 DNA was 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 using a spectrophotometer (A260/A280) and only samples with 1.8 ratios were used for further experimentation. To analyse the DNA fragmentation, electrophoresis was carried out by 0.8% agarose gel containing ethidium bromide. The gel was examined under UV light and photographed the visualized DNA bands.

III. RESULTS

ACTIVITY OF GLUTATHIONE S-TRANSFERASES (GST) AND GLUTATHIONE PEROXIDASE (GPX)

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 decreased by 53%, 34%, 13% and 6% in response to 6 mg, 8 mg, 10 mg and 12 mg. Glutathione peroxidase I (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. Glutathione peroxidase II (Selenium dependent) activity was linearly increased to remove peroxide radicals 67%, 80% in response to 2 mg and 4 mg but linearly decreased 59%, 33%, 15% and 4% in response to 6 mg, 8 mg, 10 mg and 12 mg. As shown Table.1, significant changes were observed in both GST and GPx activities (a = $p<0.01$ and b = $p<0.05$)

As shown in fig. 2, in long term exposure to acrylamide, GST activity was increased by 43% and 60% in response to 2

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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 I (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. Glutathione peroxidase II (Selenium dependent) activity was linearly increased to remove peroxide radicals 45%, 74% in response to 2 mg and 4 mg but linearly decreased 44%, 29%, 18% 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 = $p<0.05$)

In this study, gradual increase and gradual decrease can be seen in rat brain GST and GPx by both short term and long term exposure to acrylamide. In addition to transferase activity, GST exhibits peroxidase activity also. This is also known as glutathione (selenium independent) peroxidase I (GPx I). Transferase activity was increased linearly when acrylamide infiltrated into brain, at that point peroxidase activity associated with transferase activity was increased and transferase activity was decreased linearly when acrylamide concentration was increased in brain, at that point peroxidase activity associated with transferase activity was decreased. Glutathione (selenium dependent) peroxidase II (GPx II) activity was linearly increased when acrylamide infiltrated in low concentration into the brain and its activity was linearly decreased when acrylamide concentration was increased.

This is because acrylamide was produced more and more reactive oxygen species (ROS) with increased concentration. Primarily GSTs and GPx activities were increased linearly in response to low quantity of acrylamide and to remove it and also ROS generated by it. Finally, high quantity accumulation of acrylamide and uncontrollable generation of ROS destabilizes the activity of GST and GPx and there by GST and GPx activities were decreased linearly.

DNA FRAGMENTATION ANALYSIS

Rat brain DNA fragmentation was evaluated by agarose gel electrophoresis. DNA was damaged at the concentration of 12 mg in both short term and long term treatment of acrylamide. Damaged in Damaged DNA was visualized a smear like band 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 and long period accumulation of acrylamide released excessive reactive oxygen species (ROS) into brain and these ROS interact long time with DNA.

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 were 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 significant.

IV. DISCUSSION

Oxidative damage occurs due to over production of reactive oxygen species (O^2, H_2O_2 and -OH) than the antioxidant capability of the cells (Kesba & El-Belagi, 2012). Oxidative stress is one of the major factor that lead to hepatotoxicity, neurotoxicity and nephrotoxicity (Kasprzak, 2002). Generally, an endogenous antioxidant system may counteract the ROS and reduce the oxidative stress with the enzymatic antioxidants GST and GPx. Change in lipid peroxidation production reactions and antioxidant defense systems were associated with changes in a variety of biochemical pathways (Paliwal et al., 2011).

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) (Prohaska and Ganther, 1977) and our study results were similar to it. Selenium independent glutathione peroxidase activity is associated with rat liver microsomal GSTs (Reddy et al., 1981). Due to oxidation of the double bond between the α and β carbons, that the acrylamide is metabolised into a reactive epoxide i.e glycidamide in liver (Lineback et al., 2012) and this epoxide induces strand breakage by the production of DNA adducts (Watzek et al., 2012). Acrylamide induce DNA damage and generates oxidative changes in rat brain (Alturfan et al 2012; Ibrahim et al., 2015) and this was supported present study. Acrylamide is genotoxic by promoting both chromosomal clastogenesis and DNA-strand breakage (Miguel et al., 2013).

V. CONCLUSION

Both short term and long term exposure of rats to acrylamide was evidenced by the observed significant alterations in brain GST and GPx activities. So, the acrylamide was did hazardous alterations in GST and GPx activities and badly damaged DNA. The findings of this study indicate that exposure to acrylamide is capable of inducing adverse significant changes GST and GPx activities and marked harmful alterations in DNA. Key findings of this study, that the conjugation and peroxidase activities associated with GSTs towards a wide variety of electrophilic compounds, reveals that the dual activities are an integral part of the cellular antioxidant defence system and the acrylamide induced oxidative stress is associated with increased production of oxidizing species as well as decrease the efficiency of antioxidant defence system, consequently oxidative DNA damage is an inevitable. The present result may contribute to better understanding of the acrylamide induced toxicity in animals, and also may be considered as an experimental base of the relevant human studies.

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