

Full Paper

Biochemical changes in the liver of Swiss albino mice orally exposed to acrylamide

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Abstract : Acrylamide is a common chemical which is used worldwide to synthesise polyacrylamide. Polyacrylamide and acrylamide both have numerous applications in cosmetic industries, plastic and aesthetic surgeries, ophthalmic operations, waste water treatments, oil recovery processes, and other industrial and laboratory processes. Exposure of mice (*Mus musculus*) to acrylamide at three dose levels (5, 15 and 25 mg/kg body weight) was investigated for its effects on the liver. Mortality was found to be nil in all the experimental groups. A significant decrease in body weight gain and liver weight was observed but the relative liver weight increased significantly with dose concentration. A significant decrease was observed in protein and GSH levels as compared to those of control, and this reduction was more pronounced at highest dose level. Concentrations of SGOT, SGPT, and serum alkaline phosphatase activity showed a significant increase which was directly proportional to the concentration of the dose.

Keywords: acrylamide, GSH, protein, alkaline phosphatase, SGOT, SGPT

Introduction

Acrylamide ($\text{CH}_2=\text{CH}-\text{CONH}_2$) is a common chemical which is used in both industrial and laboratory processes. The most important use of acrylamide is in the production of high molecular weight polyacrylamide which is produced to give non-ionic, cationic and anionic properties for specific uses [1]. The co-polymers and polymers of acrylamide have a wide range

of applications, e.g. treatment of drinking water, waste water, soil and sand; processing of crude oil, paper, pulp, minerals, concrete, and textiles. They are also used as filler in cosmetic industry, as hydrogel in ophthalmic operations, as an ingredient of microencapsulated gelspheres for drug treatment, in plastic and such aesthetic surgeries as breast augmentation, and in contact lenses, photographic emulsions and adhesives [2-6]. In scientific researches acrylamide is also used to selectively modify –SH groups and as a quencher of tryptophan fluorescence in studies designed to elucidate the structure and functions of proteins [7].

Acrylamide occurs in both solid (crystalline) and liquid (aqueous) forms. The solid monomer is a colorless, free flowing crystal which is soluble in water, methanol, ethanol, dimethyl ether and acetone, and is insoluble in benzene and heptane. Commercially acrylamide is produced by catalytic hydration of acrylonitrile [1,8]. Recent investigations have indicated that it is also formed in heated starchy foods especially potato products via the Maillard-type reaction mainly between the amino acid asparagine and a carbonyl source such as the reducing sugars glucose and fructose [9-10]. It is also a component of tobacco smoke, which indicates that it can also be formed by heating of tobacco-containing products like cigarette, cigar, etc. [11-12]

Although the polymeric form of acrylamide is reported to be nontoxic, its monomeric form has a potential to cause a wide spectrum of toxic effects [13-17]. It is reported to be a multisite carcinogen, which can induce tumours of the adrenal, thyroid, CNS, oral cavity, testis, mammary gland and uterus [18-19]. Genotoxicity of acrylamide has also been reported, which comprised chromosomal aberrations, sister chromatid exchanges, unscheduled DNA synthesis, and dominant lethality [20-26]. Changes in copulatory behaviour, decreased fertility, effects on sperm motility and morphology in treated male, and alterations in the estrogen/progesterone balance in offsprings of treated females are the other reported toxic effects related to the reproductive toxicity of this chemical [27-28]. Acrylamide is also a neurotoxin which induces paraesthesias in fingers, coldness and weakness of hands, numbness in lower limbs, drowsiness, hallucinations, ataxia, convulsions, diffused damage to different sections of the nervous system, lysis in the cerebellum neurons and tibial nerve degeneration [29-33].

The liver plays a central role in the process of detoxification in the body. Glutathione (GSH), a tripeptide present in the liver, kidney, brain, and erythrocyte has a significant binding capacity with toxic substances resulting in the formation of usually non-reactive conjugated products [34]. A large reserve of GSH in hepatocytes makes the liver more efficient in the process of detoxification. However, if the rate of production of the toxic metabolites exceeds the availability of glutathione, hepatotoxicity can occur. Similarly, depletion of the protein store of the liver is also an indicator of hepatic disorders.

Different cells have different enzymes inside them, depending on the function of the cell. When cells die or are damaged, the enzymes leak out causing the blood level of these enzymes to rise. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase are some liver-function test enzymes, the elevation of which, in serum, reflects

some hepatic disorders [35]. Therefore, to evaluate the liver damage, estimation of these enzymes in serum is essential.

The aim of the current study is to evaluate the hepatotoxicity in Swiss albino mice orally exposed to acrylamide.

Material and Methods

General

Adult Swiss albino mice (*Mus musculus*), 8-10 weeks old with body weight of 26 ± 2 gm, were used. They were maintained and bred in animal house as outbred colony as per norms laid down by an Institutional Ethical Committee, given standard mice food and water *ad libitum*. Acrylamide monomer of 99% purity was obtained from Central Drug House (P) Ltd., Bombay, in powder form. It was dissolved in doubly-distilled water to obtain a 1% solution and kept in a dark brown bottle to avoid polymerisation. The experiment was conducted for the duration of 60 days and divided into three groups, viz. E1, E2, and E3, each with 10 animals that were given 5, 15, and 25 mg respectively of acrylamide/kg body weight. Along with these experimental groups a control group was also set, in which animals were given doubly-distilled water equal to the volume of acrylamide solution given in the experimental group of the highest dose. The dosing of control and all experimental groups was carried out orally with a gavage needle on alternate days for the duration of 60 days and animals were checked daily for any mortality. Mice were autopsied by cervical dislocation after 24 hours of oral administration of the last dose on the 61st day. The serum was obtained by collecting the blood obtained by cardiac puncture in sample bottles without anticoagulant. The blood was centrifuged for 10 minutes at 3000 rpm and the resulting serum was used for analysis. The liver were dissected out carefully, blotted free of blood, weighed, and utilised for the study.

Liver biochemical analysis

Protein concentration in the liver was determined by the method of Lowry et al.[36] and expressed as mg/100mg of tissue weight. Concentration of GSH in the liver was measured using the method described by Moron et al. [37] and expressed as $\mu\text{mol/gm}$ of tissue weight.

Serum biochemical analysis

The activity of alkaline phosphatase in the serum was measured using the method of Fiske and Subarrow [38] and expressed as mg Pi/gm/h. Determination of serum transaminases (SGOT and SGPT) was carried out by the method of Reitman and Frankel [39] and expressed as IU/L. The collected data were statistically analysed by student's t- test and the treatment groups were considered statistically significant at $P < 0.001$, $P < 0.01$ and $P < 0.05$.

Results and Discussion

The mortality rate was observed to be nil in all the experimental groups. A significant reduction ($P < 0.001$) in body weight gain and liver weight ($P < 0.01$ in E1 and E2, $P < 0.001$ in E3) was observed but the relative liver weight [= (liver weight/body weight) $\times 100$] increased non-significantly in E1 and E2 and significantly ($P < 0.05$) in E3 [Table 1]. Protein and GSH levels in liver decreased significantly ($P < 0.001$) in comparison to those of control [Table 2]. Serum SGOT and SGPT concentration and alkaline phosphatase activity showed a significant increase ($P < 0.001$) as compared to control, the increase being directly proportional to the concentration of acrylamide dose [Table 3].

Table 1. Changes in body weight and liver weight of Swiss albino mice

Experimental group	Initial body weight (g)	Final body weight (g)	Body weight (g)	Liver weight (g)	Relative liver weight*
Control	26.77 \pm 0.281	33.22 \pm 0.496	6.556 \pm 0.525	2.402 \pm 0.056	7.234 \pm 0.218
E1	26.98 \pm 0.815	27.85 \pm 0.774	0.876 \pm 0.233 ***	2.105 \pm 0.060**	7.564 \pm 0.356 NS
E2	26.54 \pm 0.287	27.14 \pm 0.326	0.601 \pm 0.189 ***	2.078 \pm 0.060**	7.658 \pm 0.236 NS
E3	26.65 \pm 0.707	25.58 \pm 0.344	-1.065 \pm 0.534 ***	2.035 \pm 0.033***	7.957 \pm 0.192*

* = [liver weight/body weight] \times 100

Note: Values are depicted as Mean \pm SEM. Significance level: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS (not significant)

Table 2. Changes in protein and glutathione (GSH) level in liver of Swiss albino mice

Experimental group	Protein (mg/100mg tissue weight)	GSH (μ mol/gm tissue weight)
Control	28.449 \pm 0.372	86.465 \pm 0.364
E1	16.531 \pm 0.534 ***	45.455 \pm 0.418 ***
E2	12.589 \pm 0.296 ***	40.522 \pm 0.577 ***
E3	8.715 \pm 0.409 ***	34.352 \pm 0.377***

Note: Values are depicted as Mean \pm SEM. Significance level: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS (not significant)

Table 3. Changes in alkaline phosphatase (ALP) activity, and SGOT and SGPT concentration in serum of Swiss albino mice

Experimental group	ALP (mg Pi/gm/h)	SGOT (IU/L)	SGPT (IU/L)
Control	5.633± 0.252	29.59 ± 0.260	21.03 ± 0.599
E1	11.699± 0.222 ***	43.647 ± 0.723 ***	32.935 ± 0.565 ***
E2	14.492 ± 0.380 ***	48.102 ± 0.740 ***	35.731 ± 0.453***
E3	17.98 ± 0.724 ***	54.102 ± 0.346***	39.148 ± 0.456 ***

Note: Values are depicted as Mean ± SEM. Significance level: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, NS (not significant)

The steady decrease in hepatic protein level with higher doses of acrylamide can be attributed to retarded protein synthesis, or to any change in protein metabolism, or to the leaking out of protein reserves from hepatocytes. Previous findings have shown that acrylamide and its metabolite glycidamide have an affinity to bind with DNA and can cause chromosomal aberrations [22-23,40]. Thus, any abnormality in DNA structure can affect transcription and ultimately protein synthesis. As reported [7,41], the acrylamide molecule has two reactive sites, viz. the conjugated double bond and the amide group. Therefore it can conjugate with the –SH group of a sulfur amino acid, the α -NH₂ group of a free amino acid, the ϵ -NH₂ group of lysine, the ring NH group of histidine, and the N- terminal residue of a protein. The above scenario can explain the unavailability of a few amino acids for protein synthesis, which might consequently be a reason for the depleted protein content in the liver. Further, being an electrophilic compound, acrylamide can bind with proteins that also can make them undetectable. Hepatocellular necrosis can also lead to the leaking out of protein reserve from the liver to the blood, thereby reducing the protein concentration of the liver.

The low levels of GSH in the present study might be due to the strong affinity of the –SH group of sulfur amino acids like cysteine and methionine for the double bond of such conjugated vinyl compounds as acrylamide, which might have reduced the concentration of these amino acids which are the main components of GSH. Further, the oxidative stress due to acrylamide might also result in oxidation of GSH to GSSG, hence decreasing the GSH concentration. Other studies have suggested that the conjugation of acrylamide to GSH catalysed by glutathione-S-transferase (GST) and excretion as mercapturic acid is a major pathway for the metabolism and detoxification of the same [42-44]. Since a large reserve of GSH is present in the hepatocytes, therefore in the process of detoxification of acrylamide the utilisation of liver GSH would be more pronounced, and thus the detoxification mechanism can also be the cause of the decreased concentration of GSH. Other studies have also shown a significant decrease in protein synthesis and GSH level in neuroblastoma cells on exposure to acrylamide [45-46].

A gradual increase in serum alkaline phosphatase (ALP) activity and also SGPT and SGOT level in this study could be due to the bipolar nature of acrylamide, where the CH₂=CH- part may undergo hydrophobic interactions while the -CONH₂ part can form hydrogen bonds with the cell components. This property may enhance its ability to alter the cell membrane structure and make the parenchymal cell membrane of the liver more permeable, thereby ceasing the active retention of enzymes and making them appear first in the extracellular space and then in the blood. These results obtained in the current study are in agreement with other studies which also indicated an increase in activity of the liver enzymes following liver damage in fish and albino mouse [47,48].

Conclusions

The available information based upon human epidemiological and population-based studies on the adverse effects of acrylamide confirms its neurotoxicity, but due to the low statistical power and the limited range of exposure doses, other effects such as reproductive toxicity, genotoxicity and carcinogenicity are still on the verge of potential human health risks. Since absence of evidence is not the evidence of absence, therefore continuing research is needed for the development of models aimed at extrapolating human health risks and the above elementary study is a small step in this direction.

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Mj. Int. J. Sci. Tech. **2008**, 2(03), 542-550

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