Acrylamide-induced genotoxic, biochemical and pathological perturbations in male rats liver

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ABSTRACT: Acrylamide (AC) (CH_2=CH–$CONH_2$) is a common chemical which is used in both industrial and laboratory processes. It is formed in heated starchy foods especially potato products . Glycidamide (GA) is thought to be the active metabolite that playing a central role in AC genotoxicity and carcinogencity . The aim of the present study was to clarify the possible involvement of genotoxic mechanisms in acrylamide-induced carcinogenicity and hepatotoxicity by measuring the role of CYP 450 2E1 (gene protein and mRNA) in rats intoxicated with AC and recording the DNA damage in their hepatic lobes by the in vivo alkaline single cell gel electrophoresis (comet assay). Thirty mature male albino rats were used in this study. Rats were classified randomly into three groups; the first group daily received 50 mg/kg AC orally for 21 days **.** The second group received twice the first dose (100 mg/kg) by the same route and duration and the third group was kept as control. The results of the present study revealed that , acrylamide caused marked alterations in animal behaviour and early mortality level that was noticed in the treated group which reached 31% (in low dose group) and 38% (in high dose group) respectively. It elicited a significant ($P<0.05$) increase in serum AST and ALT, and a significant decrease of total protein, albumin and globulin levels. The immune-blot of CYP2E1 protein revealed that, the protein level of CYP2E1 in acrylamide treated rats was significantly down regulated. Also it caused down regulation of gene mRNA expression of CYP 2E1. A statistically significant increase of the number of DNA single strand breaks was evident with Comet assay. Histopathological investigations of liver of AC treated rats showed necrotic changes represented by granular eosinophlic cytoplasm and karolysis of some nuclei, round cell infiltration in portal and interstitial tissue beside telangiectiasis in some hepatic sinusoids, also degenerative changes were observed which characterized by cloudy swelling or hydropic degeneration of some hepatic cells with hypotrophied kupffer cells. Finally , we recommend restriction of acrylamide exposure either occupationally or in food containing product in addition, raising awareness of people about its hazards.

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INTRODUCTION

Contaminants are a vast subject area of food safety and quality and can be present in our food chain from raw materials to finished product **(Erkeoglu and Baydar, 2010)**. Acrelamide (AC) is one of the most important contaminant in the environment. AC was shown to be a neurotoxicant, reproductive toxicant and carcinogen in animals. **(El-Assouli, 2009)** .

Acrylamide is an alpha,beta- unsaturated vinyl monomer of poly acrylamide (conjugated) reacted molecule . The co-polymers and polymers of AC have a wide range of applications, it is used in biochemistry , paper manufactures , waste water treatments and used as soil stabilizers, also, it is used world wide to synthesize polyacrylamide. Both polyacrylamide and AC have numerous applications in cosmetic industries, plastic and aesthetic surgeries, ophthalmic operations , oil recovery processes and other industrial and laboratory processes **(Klaunig and Kamendulis,2005 & Asha et al.,2008 and Schwend et al.,2009).**

Acrylamide is generated by thermal processing of food depending on processing condition and precursor availability. Dietary Acrylamide is largely derived from heat – induced reactions (Maillard reaction) between the predominantly amino group of the free amino acid precurser asparagine and carbonyl groups of glucose and fructose during heat processing (baking, frying) of plant-derived foods such as potato fries and cereals. After consumption, Acrylamide is absorbed into the circulation and is then distributed to various organs, where it can react with DNA, neurons, hemoglobin, and essential enzymes

(Baum et al.,2008 & Rayburn and Friedman , 2010).

The Acrylamide monomer can cause several toxic effects ; it is an animal carcinogen and germ cell mutagen present at low (ppm) levels in heated carbohydrate –containing food stuffs **Ghanayem et al., (2005)** , also it is a well known human neurotoxic agent a suspected carcinogen and has potential for human exposure either through the environment or from occupational exposure **(Klaunig and Kamendulis , 2005 and Nuno et al., 2008).**

The Acrylamide is not genotoxic by it self but becomes activated to its primary epoxoide genotoxic metabolite glycidamide (GA) via epoxidation **(Baum et al.,2008) .**

Ghanayem et al, (2005) demonstrated that CYP2E1 is the primary enzyme responsible for the epoxidation of AC to GA, which leads to the formation of GA-DNA and hemoglobin HGB adducts.

Previous important findings have refocused interest in this compound , especially because appreciable amounts of acrylamide are formed in western diets which has prompted renewed interest in its potential toxicity, so the aim of this study was to clarify the possible involvement of genotoxic mechanisms in acrylamide-induced carcinogenicity and hepatotoxicity by measuring the role of CYP 2E1 (gene protein and its mRNA) in rats intoxicated with AC and recording the DNA damage in their hepatic lobes by the *in vivo* alkaline single cell gel electrophoresis (comet assay).

MATERIAL AND METHODS

Chemical compound: Acrylamide (C_3H_5NO>99%purity) purchased from Sigma Chemical Company

Synonyms: 2- propenamide; acrylic acid amide; acrylic amide; acrylamide monomer; acrylamide; propenoic acid amide ; vinyl amide ; ethylene carboxamide

Molecular formula: C_3H_5NO

Chemical formula : $CH_2CHCONH_2$

chemical structure:

$$CH_2=CH-\underset{\underset{O}{\|}}{C}-NH_2$$

Animals and dosing

Thirty mature male albino rats with an average body weight ranging from 160-180 g were obtained from the animal research unit of the faculty of Vet. Medicine Zagazig University. Animals were kept in metal cages during the whole experimental period under hygienic conditions, fed on well balanced ration and provided with water *ad- libtum*, through the experiment. Rats were divided into three equal groups the first group daily received 50 mg/kg AC orally for 21 days**, (Sunner et al, 1999)**. The second group received twice the first dose (100 mg/kg) by the same route and duration and the third group was kept as control. The experiment was terminated after 21 days from 1st administration, where the animals were sacrificed.

Biochemical analysis

Blood samples were collected from medial canthus of the eyes of all male rats in plain tubes for serum separation according the method of **Renwick (1989).** Serum samples were analyzed for estimation of alanine aminotransferase (ALT) and the aspartate aminotransferase (AST) activities **(Bergmeyer,et al. 1978).**, total proteins **(Coles 1986)** and albumin **(Doumas et al. 1971),** were determined Serum globulin was calculated by substraction of albumin from total protein.

Microsomal preparation

The liver tissues were homogenized in 3 volumes of 1.15% potassium chloride solution and centrifuged at 9,000 g for 20 min. The supernatant fraction was centrifuged at 105,000 g for 70min**.** The washed microsomes were then suspended in 0.1M potassium phosphate buffer, pH7.4 **(Omura and Sato 1964).** Microsomal protein concentrations were determined by the method of **Lowry (1951)** using bovine serum albumin (Sigma Chemical Co.) as a standard.

Western immunoblotting analyses of CYP2E1 protein

Liver microsomes (5mg protein) were electrophoresed (Mini-Protean II, Bio-Rad Lab., Richmond, CA) through 10% Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) slab gels **(Towbin et al., 1979).** Resolved proteins were transferred to nitrocellulose Trans-Blot membranes (Bio-Rad Laboratories, Hercules CA). The blots were stained with Ponceau S to confirm that protein content is approximately the same in all lanes. Filters were soaked in phosphate buffer solution, pH7.5 (PBS) to remove the Ponceau S and were incubated overnight in PBS containing 5% dried skimmed milk and 0.1% Tween-20, to block excess protein binding sites. The membranes were then incubated with a primary goat polyclonal anti-rat CYP2E1 antibody (Gentest Co., Woburn, MA), detected with horseradish peroxidase-conjugated secondary antibodies and enhanced chemiluminiscence reagents (Amersham Pharmacia Biotech, Ltd, Buckinghamshire, UK), and visualized. The CYP2E1 content was quantified by analysis

densitometrically scanned using NIH image software 1.61.

RNA extraction

Total RNA was isolated from 50mg of liver using Trizol reagent (Life Technologies Inc, Grand Island, NY, USA). Briefly liver tissue samples were homogenized in 1ml of Trizol then 0.3ml of chloroform was added to the sample. The mixtures were then shaken for 30sec followed by centrifugation at 4°C and 15,000g for 20min. The supernatant layer were transferred to a new set of tubes, and an equal volume of isopropanol was added to the samples, shacked for 15sec and centrifuged 4°C and 15,000g for 15min. The RNA pellets were washed with 70% ethanol. RNA was dissolved in DEPC water. The prepared RNA was checked by electerophoresis, and showed that the RNA integrity was fine, and then it was further checked by measuring the optic density (OD) on specterophotometer. The OD of all RNA sample was 1.7 to 1.9 based on the 260/280 ratio.

Semi-quantitative RT-PCR

A mixture of 5µg of total RNA and 0.5ng oligo dT primer in a total volume of 24µl sterilized ultra-pure water, was incubated at 70 ºC for 10min and then removed from the thermal cycler and completed to 40µl with a mixture of 8 µl (5X) RT-buffer, 2µl 10mM dNTP, 2µl DEPC water, and 2µl of reverse transcriptase (Toyobo CO., Ltd., Osaka, Japan) and incubated in the thermal cycler at 30 ºC for 10min, 42ºC for 1h, and 90 ºC for 10min. For PCR, 1µl aliquots of the synthesized cDNA were added to 20µl of a mixture containing sterilized ultra-pure water, 2µl of PCR buffer, 2µl of dNTP (2.5mM), 0.3µl of sense and anti-sense primers (10µM) and 0.1µl of Taq polymerase (Takara, Kyoto, Japan). Specific CYP2E1 primers were designed according to **Gonzalez et al. (2003)**, the sequence: GAAAAAGCCAAGGAACACC (sense) and GCAGACAGGAGCAGAAACA (antisense) as published PCR For semi-quantitative RT-PCR assays, a co-amplification approach was used with glyceraldehyde-3-phosphate dehydrogenase (G3PDH) as the control gene. Rat liver and the PCR conditions were the same as described above, except that 10pmol G3PDH primers (sense): TGAAGGTCGGTGTGAACGGATTTGGC and (antisense): CATGTAGGCCATGAGGTCCACCAC. Amplification was initiated by denaturation of 1 cycle at 95ºC for 1min followed by denaturation at 94ºC for 1min, and annealing at the proper temperature for 1min then extension at 72ºC for 1min for the proper number of cycles for each gene using a DNA thermal cycler (BioRad, Hercules, CA, USA). The samples were finally incubated for 7min at 72ºC after the last cycle of amplification. The amplified PCR products were separated by electrophoresis through 1-1.5% agarose gel. Bands of cDNA were stained with ethidium bromide and visualized by ultraviolet illumination. The CYP2E1 and G3PDH contents were densitometrically scanned using NIH image software 1.61. G3PDH mRNA levels were used for the correction of CYP2E1 mRNA expression as endogenous genes; the ratio between CYP2E1 and G3PDH was determined by densitometry.

Comet assay

Five animals of each experimental group were sacrificed and a small piece of the left hepatic lobe was collected and placed onto a small Petri dish with ice-cold mincing solution (Ca^{2+}- and Mg^{2+}-free HBSS containing 20 mM EDTA and 10% DMSO). The viability of the liver cells was indirectly determined by analyzing the comet images after electrophoresis **Endoh et al, (2002)**. The liver samples were cut into smaller pieces, using a disposable microtome razor blade, and the solution was aspirated. Then, a fresh mincing solution was added and the liver samples were minced again to finer pieces. Resulting cell suspensions were collected and filtered (100 µm nylon mesh). All samples were stocked on ice in appropriate conditions to avoid light until the comet assay procedures. The quantity of liver cells in the cell suspensions was determined in Giemsa-stained smears.

The comet assay was performed under alkaline conditions according to a previously described standard protocol **Collins and Dunsinka, (2002)**, Briefly, an aliquot of 5 µl of each prepared hepatic cell suspension was mixed with 120 µl of 0.5% low melting point agarose at 37°C and layered onto conventional microscope slides, precoated with 1.5% normal melting point agarose. The slides were placed overnight in freshly prepared cold lysing solution (1% Triton X-100, 2.5 mM NaCl, 0.1 mM Na_2EDTA, 10 mM Tris with 10% DMSO, pH 10.0) and then in a horizontal electrophoresis cube with alkaline electrophoresis solution (0.3 M NaOH, 1 mM Na_2EDTA, pH >13) at 4°C for 20 min. The electrophoresis was performed at 25 V and 300 mA for 20 min. After electrophoresis, the slides were washed twice for 5 min in neutralizing buffer (0.4 M Tris-HCl, pH 7.5), fixed for 5 min in absolute alcohol, air-dried, and stored at room temperature. In order to evaluate extremely low molecular weight DNA diffusion, two slides from each animal were removed after lysis procedure, rinsed with neutralizing solution, fixed and air-dried, and stored until analysis.

Immediately before analysis, the DNA was stained with 50 µl of 20 µg/ml ethidium bromide. The slides were examined with a 40X objective lens with epi-illuminated fluorescence microscopy (Olympus-

Bx60, excitation filter: 515-560 nm; barrier filter: 590 nm) attached to a color CCD video camera and connected to an image analysis system (Comet II, Perspective Instruments, UK). Coded slides were scored blindly and 100 hepatic cell images were randomly analyzed for each animal (50 images per slide).

The comets were analyzed by a visual scoring method and computerized image analysis. The comets were classified into five categories, defined as types 0, 1, 2, 3 and 4 - where 0 indicates no or very low damage, 1, 2 and 3 indicate low, medium and Long DNA migration, respectively, and 4 indicates apoptotic or necrotic DNA migration. Based on the extent of strand breakage, cells were classified according to their tail length in five categories, ranging from 0 (no visible tail) to 4 (still detectable head of the comet but most of the DNA in the tail). The following formula **Liu et al, (2002)** was used to calculate scores in which N is the number of cells in each category (e.g. N3 is the number of cells in category 3).

Score= (N0+N1+2xN2+3xN3+4xN4)/ (N0+N1+N2+N3+N4)

Experiments were done in duplicate and repeated at least twice.

Histopathological examination

Specimens from liver of all experimental groups were preserved and fixed in neutral-buffered formalin. Then specimens were routinely processed and sectioned at 4-5um thickness. The obtained sections were stained with H&E according to **Horobin, and Bancroft , (1998)**.

Statistical analysis

The results were analyzed using the statistical package of social science (SPSS) **version 10 software**. One way analysis of variance

(ANOVA) for comparison between more than two groups. Least significant difference (LSD) for multiple comparison **(Norusis 1997).**

RESULTS

Clinical signs :

In our experiment, administration of acrylamide to male rats resulting in marked alterations in behaviour, revealing nervous manifestations (abnormal neurobehavior) in the treated groups as ataxia, increased landing of the limbs, weakness of the muscles, general emaciation. The severity of the clinical signs was dose and time dependant.

Mortality & post-mortem appearance

The animals of the present study revealed early mortality level that was noticed in the treated groups which reached 31% (in low dose group) and 38% (in high dose group) respectively. P.M. lesions of either dead or sacrificed rats revealed sometimes generalized swelling and paleness of body organs.

Serum biochemical parameters

Rats administered Acrylamide (50 and 100mg/kg) for 21 days elicited a significant ($P<0.05$) increase in serum AST and ALT activities, this increase was dose dependent. On the other hand both treated groups revealed significant decrease of total protein, albumin and globulin levels comparing with control group (Table 1).

Expression level of CYP2E1 protein

On measuring the expression level of CYP2E1 the immune-blot of CYP2E1 protein revealed that, the gene protein of CYP2E1 in acrylamide treated rats was significantly down regulated and this inhibition was dose dependent, (Figure 1A-B). In addition, our results also revealed that acrylamide significantly downregulated mRNA expression of CYP2E1 in a dose-dependent manner in comparison to control group (Figure 2A-B).

Comet assay

The role of AC on the direct DNA single strand (ss) breaks was evaluated with Comet assay which could detect DNA ss breaks in hepatocytes after treatment. A statistically significant increase of the number of DNA ss breaks was evident with both examined concentrations of Ac and this increase was dose dependent **(Figure 3)**

Histopathology findings

The examined livers of AC treated rats with a dose of 50 mg /kg b.wt. (low dosed group) showed mild reversible degenerative changes characterized by cloudy swelling or hydropic degeneration of some hepatic cells , hypotrophied Kupffer's cells together with dilated and congested blood vessels and hepatic sinusoids beside numerous bile ductules were seen (Figure 4**).** Some portal areas exhibited edema and proliferative biliary epithelium with round cell infiltration. While AC treated rats with a dose of 100 mg /kg b.wt. (high dosed group) showed mild necrotic changes in the hepatic parenchyma represented by granular eosinophlic cytoplasm and karyolysis of some nuclei together with portal mononuclear cell infiltration (Fig.5). Moreover, round cell infiltration could be seen in portal and interstitial tissue beside telangiectiasis in some hepatic sinusoids.

Table 1. Effect of acrylamide on serum ALT,AST, total protein, albumin and globulin levels, AC was orally administered daily for 21 days by two doses levels 50 and100mg/kg b.wt.

Parameter / Group	AST (UL)	ALT (UL)	Total protein (gm/dl)	Albumin (gm/dl)	Globulin (gm/dl)
Control	34.6±2.3	23.8±1.7	6.9±0.3	4.1±0.1	2.8±0.2
AC low dosed group (50mg/kg)	42.8±1.9*	32.2±2.2*	5.3±0.6*	3.2±0.4*	2.1±0.2*
AC high dosed group (100mg/kg)	58.6±3.4**	37.6±1.6**	4.5±0.3**	2.3±0.2**	2.2±0.1*

*Significantly different from control ** highly significantly different from control

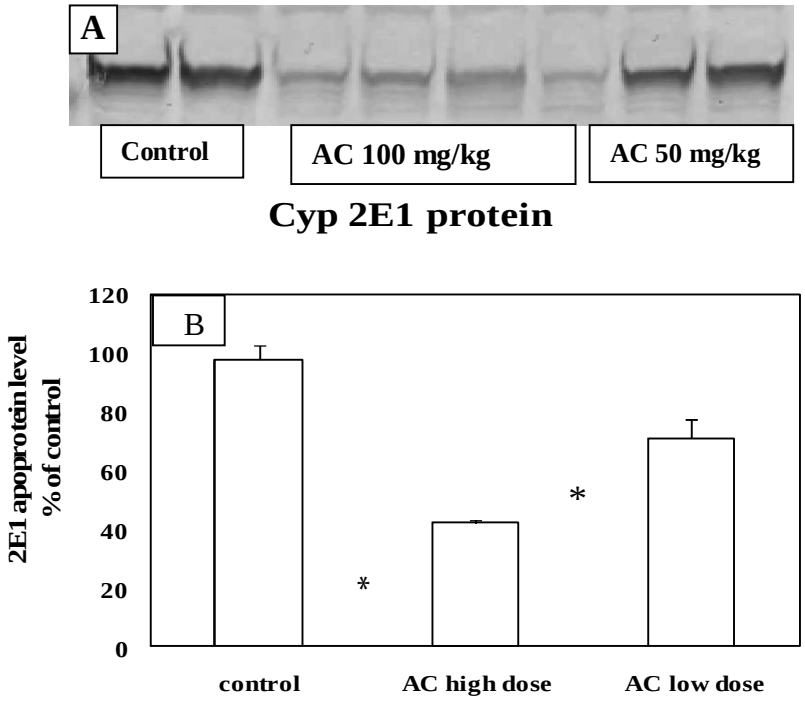

Figure1 (A-B): Effects of acrylamide administration on expression levels of CYP2E1 protein, AC was orally administered daily for 21 days by two dose levels 50 and 100mg/kg.
*$p < 0.05$ was considered statistically significant compared to control group.

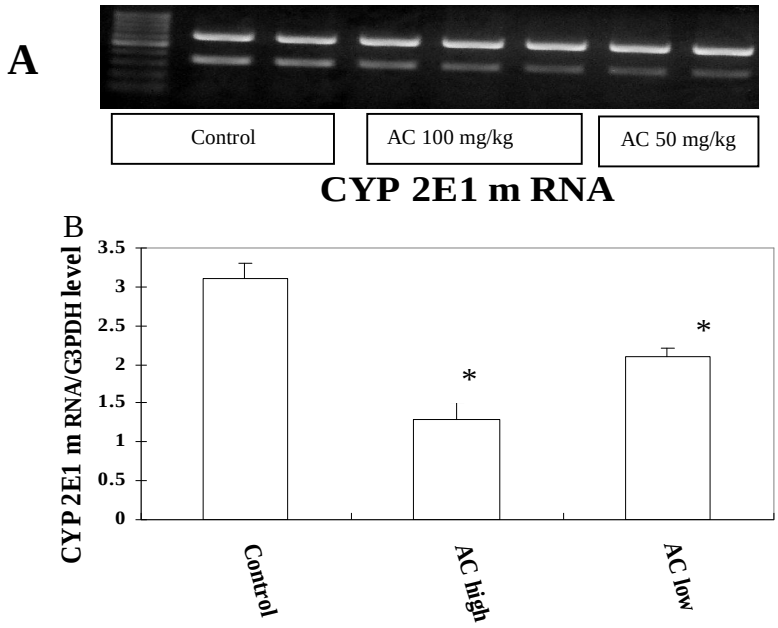

Figure 2(A-B) Effects of oral administration of acrylamide on Semi-quantitative CYP2E1 mRNA expression in the liver of control and AC treated rats throughout period of the study. The values of CYP2E1 expression were corrected to G3PDH expression levels. *p < 0.05 was considered statistically significant compared to control group

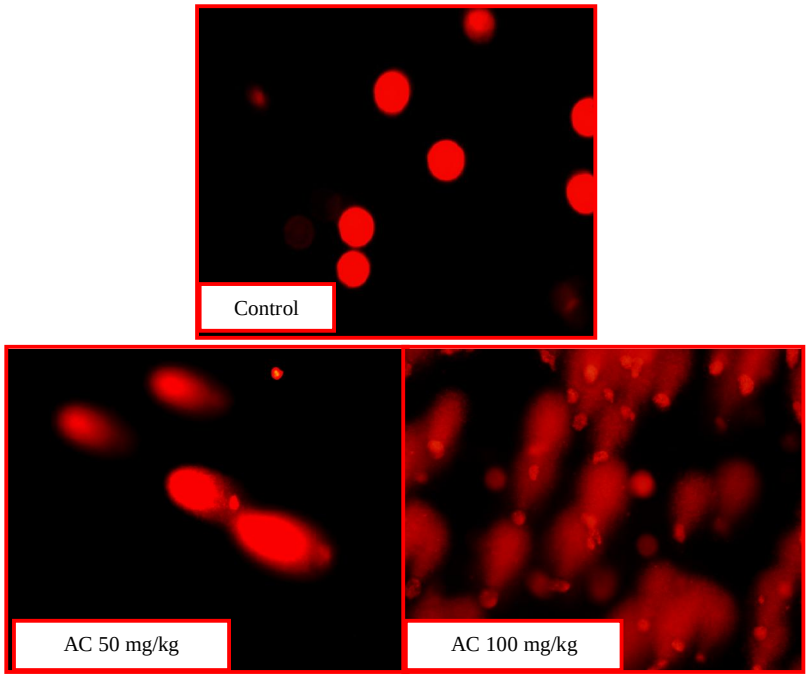

Figure 3. Comet images of liver cells treated with:
Control : PBS treated cells.
AC 50mg: hepatocytes from rats treated with Acrylamide 50mg/kg daily for 21 days.
AC-100 hepatocytes from rats treated with Acrylamide 100 mg/kg daily for 21 days

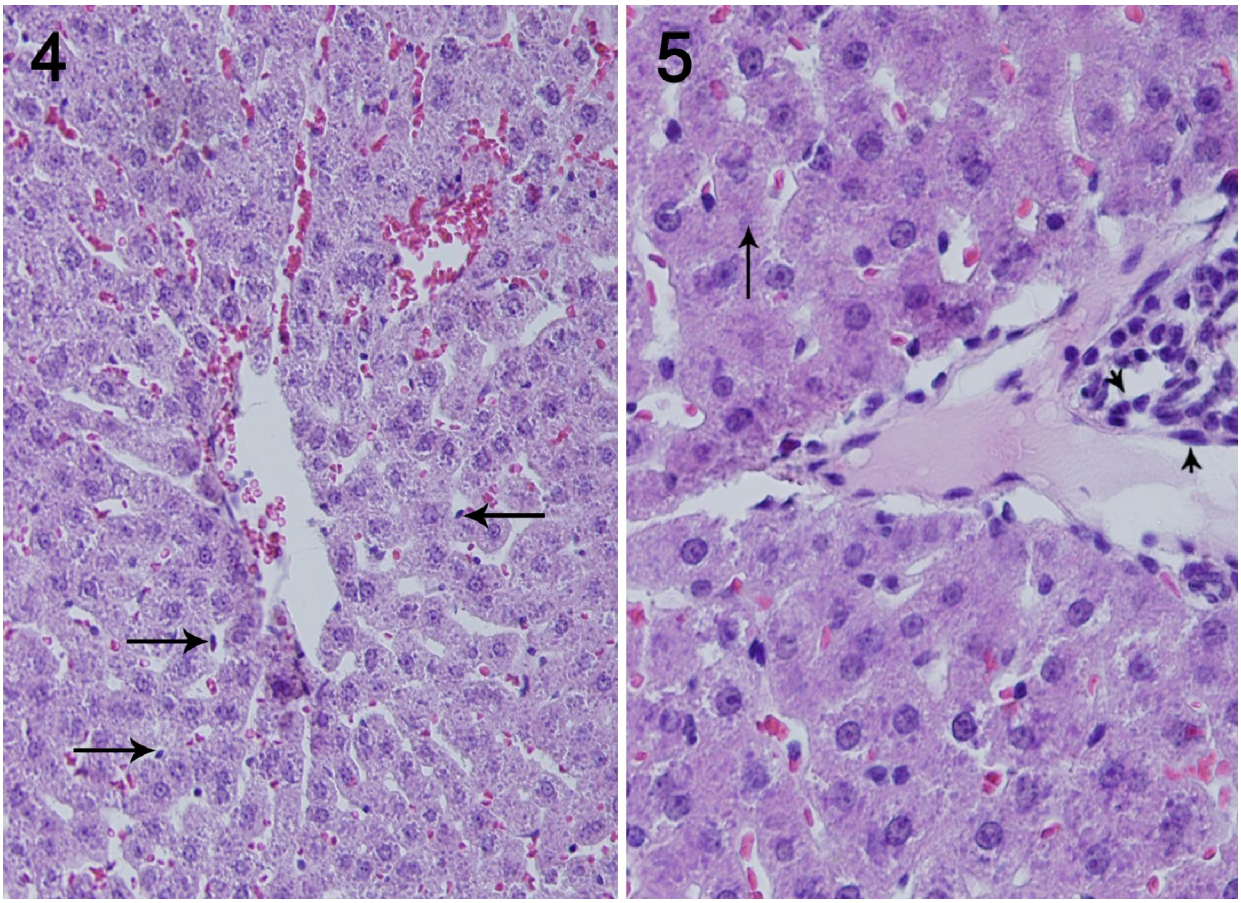

Fig. 4. Section in liver of male of rat orally administered acrylamide daily at dose of 50 mg/kg b.wt for 21days showing degenerative changes characterized by cloudy swelling or hydropic degeneration of some hepatic cells, hypotrophied Kupffer cells (arrow) (H & E X 300) .

Fig. 5. Section in liver of male of rat orally administered acrylamide daily at dose of 100 mg/kg b.wt for 21days showing mild necrotic changes in the hepatic cells (arrow) with focal mononuclear cells infiltration (head of arrow) in portal area (H & E x 1200).

DISCUSSION

Acrylamide is a water-soluble vinyl monomer that is primarily used for production of polymers that have abroad applications to various chemical industries, e.g. water and wastewater management, ore processing and dye synthesis. In addition, AC is also used extensively in molecular laboratories for gel chromatography and is present in certain foods prepared at very high temperatures **(Shan et al., 2006).**

Acrylamide monomer may form in certain foods cooked at high temperatures. The highest concentrations of acrylamide have been identified in potato and grain-based foods that are cooked at very high temperatures (e.g., frying, grilling or baking) **(Tareke et al. 2002**).

The present study revealed that oral intubation of acrylamide to rats at concentrations of 50 and 100mg/kg for 21 days induced deleterious effects and marked alterations in the determined parameters, it is believed that the reasons of the high mortalities that observed in our work could be attributed to AC toxicity, hind limb dysfunction and abnormal gait led to inability to get food, in addition to AC may caused alterations in thirst and hunger regulation centres in hypothalamus **(WHO, 1985).**

Rats administered Acrylamide (50-100mg/kg) for 21 days elicited a significant ($P<0.05$) increase in serum AST and ALT activities this increase was dose dependent. These results came in harmony with the hypothesis recorded by **(Dheer et al., 1987, Chinoy and Memon , 2001)** who stated that the significant increase in serum AST, ALT levels could be due to the

bipolar nature of acrylamide, where the CH2=CH part may undergo hydrophobic inter actions 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 liver more permeable, thereby ceasing the active retention of enzymes and making them appear first in the extracellular space and then in the blood.

Yousef and El-Demerdash (2005) reported significant elevation of these enzymes in plasma post acrylamide intoxication. They attributed these results that, biochemical parameters are sensitive index to changes due to xenobiotics and can constitute important diagnostic tool in toxicological studies.

Transaminase and phosphatases are important and critical enzymes in biological processes, they are responsible for detoxification, metabolism and biosynthesis of energetic macromolecules for different essential functions. Any interference in these enzymes leads to biochemical impairment and lesions of the tissue and cellular function **(Khan et al., 2001).**

The increased serum levels of aminotransferases in our study have been attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into circulation after AC-induced cellular damage which manifested histologically by our histopathological findings which revealed necrotic changes represented by granular eosinophlic cytoplasm and karolysis of some nuclei, round cell infiltration in portal and interstitial tissue beside telangiectiasis in some hepatic sinusoids, also degenerative changes were observed which characterized by cloudy swelling or hydropic degeneration of some hepatic cells with hypotrophied kupffer's cells (Figure 4, 5).

Both serum and histological findings may be contribute to protein degradation which manifested by the low level of total protein, albumin and globulin in our result. The obtained results were found to be supported by the results of **Asha et al. (2008**) who reported that there were steady decrease in hepatic protein level with higher doses of acrylamide which can be attributed to retarded protein synthesis, or to change in protein metabolism or to the leaking out of protein reserves from hepatocytes. 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 acids , the α-NH2 group of a free amino acid, the –NH 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 acid for protein synthesis, which might consequently be areason for the debilitated protein content in the liver. Further being an electrophilic compound acrylamide can bind with protein that also can make them undetectable. Our histopathological results (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.

Cytochrome P450 (CYP), a superfamily of hemoproteins involved in the metabolism of numerous xenobiotics with unrelated chemical structures comprises several isoforms with overlapping substrate specificity. Many of these substrates form reactive intermediates, thus becoming potent toxicants, mutagens or carcinogens. Among these is CYP2E1, an isoform involved in the biotransformation of several small organic chemicals, including ethanol, acetone, pyridine, nitrosamines, CCL4, acrylamide and many others. The regulation of CYP2E1 by some of these chemicals is highly complex**, (Jasso et al, 2003)**

Treatment of male albino rats with both doses of acrylamide for 21 days down regulated hepatic CYP2E1 protein which postulate that acrylamide requires CYP2E1-associated bioactivation to produce its active metabolite glycidamide and so liver injury. The hepatic microsomes from control, AC treated rats were resolved by SDS–PAGE and immune-blotted with anti-CYP2E1. The immune-blot of CYP2E1 protein revealed that, the protein level of CYP2E1 in acrylamide treated rats was significantly down regulated and this inhibition was dose dependent, (Figure 1A-B). These observation is in accord with the kinetic data, which showed that CYP2E1 is the rate- limiting factor of metabolic activation of AC to glycidamide (**Tareke et al., 2002, Hazardous Substances Data Bank, 2003& PHS, Public Health Service ; 2004)** The down regulation of CYP2E1 gene protein was accompanied by reduction of the expression of CYP2E1 mRNA in a dose – dependent manner in comparison to control group (Fig. 2A-B). This down regulation may be due to direct cytotoxicity of the glycidamide (GA) which leads to reduction in the expression of CYP2E1 protein and mRNA due to inhibition of its transcription from the damaged hepatocyte. In this direction our results are supported by the findings of **Naoki et al., (2010)** who reported that the down regulation of CYP2E1 may indicates and confirm its role in the metabolism of AC as it was reported that CYP2E1 is predominantly active at high AC concentrations.

Previous findings have shown that AC and its metabolite GA have affinity to bind with DNA, thus any abnormality in DNA structure can affect transcription and ultimately protein synthesis **(Dearfield et al., 1995**, **CERHR; Center for**

Evualtion of Ristks To Human Reproduction , 2004 & **Husoy et al., 2005)** Substantial genotoxicity in the three types of cell tested (V79, Caco-2, and primary rat hepatocytes) with highly significant induction of DNA strand breaks were recorded by **Nicole et al (2005)**. The parent compound AC was found to possess only weak DNA damaging properties in V79 and Caco-2 cells, detectable as DNA strand breaks in the comet assay. Only at the highest concentration (6mM AA, 24 h) DNA strand breaks were induced, without substantial differences in sensitivity between these cell lines of different species origin. However, V79 and Caco-2 cells lack substantial expression of CYP2E1. Therefore, the conversion of AC to the more reactive GA is not expected to occur effectively in these cells

So our experiment conducted to measure the DNA peroxidative damage in rats liver *(in vivo*) to insure the role of CYP2E1 and so glycidamide in the AC genotoxicity.

The present study evaluated the role of AC on the direct DNA single strand (ss) breaks with Comet assay, which could detect DNA ss breaks in hepatocytes immediately after treatment. A statistically significant increase of the number of DNA ss breaks was evident with both examined concentrations of AC and this increase was dose dependent (Figures 3).

We postulated the obtained strand breaks was mainly due to glycidamide; acrylamide reactive metabolite which ensured by its dose dependency. **Schwend et al. (2009)** stated that AC ; alpha , beta – unsateurated vinayl monomer causes cytoxicity due to its alkylating properties.

Several adducts of glycidamide with the purine bases of DNA have been described as supralinear dose–response relationship and their appearance in DNA of liver, lung and kidney of mice treated with acrylamide has been demonstrated, which is consistent with saturation of oxidative biotransformation of acrylamide at higher doses **(Gamboa da Costa et al, 2003)**. On the same context those observations were recorded by **Besaratinia and Pfeifer (2004)**who concluded that the mutagenicity of acrylamide in human and mouse cells is based on the capacity of its metabolite, glycidamide, to form pro-mutagenic DNA adducts.

Also, **Nicole et al., (2005)** reported that acrylamide (AC) possess clastogenic and mutagenic properties *in vivo* However, AC itself is known to react quite slowly with DNA. Therefore, its metabolite GA, formed by CYP2E1, is proposed to represent the ultimate carcinogen. GA is known to be clastogenic and mutagenic in vitro and in vivo. There is substantial evidence that glycidamide (GA), metabolically formed from AC by CYP2E1-mediated epoxidation, acts as the ultimate mutagenic agent.

Besides, our results are coincide with the attribution reported by **Ghanayem et al., (2005)** who clearly implicating that CYP2E1-mediated formation of GA in the induction of genetic damage in the male germ cells, they hypothesized that AC induced somatic cell damage which is also caused by GA. Their result supported the hypothesis that genetic damage in somatic and germ cells of mice treated with acrylamide is dependant upon metabolism of the parent compound by CYP2E1. This dependency on metabolism may result in altered metabolic efficiency leading differential susceptibilities.

Conclusion:

Acrylamide caused marked alterations in animal behaviour and early mortality level**,** it elicited a significant increase in serum AST and ALT, and a significant decrease of total protein, albumin and globulin levels, these biochemical changes coincide with histopathological alteration in liver tissues **.** The expression level of both CYP2E1 gene protein and gene mRNA were significantly down regulated. A statistically significant increase of the number of DNA single strand breaks was evident with Comet assay. All recorded changes in all studied parameters were dose-dependent. So we recommend restriction of acrylamide exposure either occupationally or in food containing product in addition, raising awareness of people about its hazards.

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الملخص العربي

الأكر لام د حدث تسمم وراثي وخلل ك م ائي ح وي ونس جي في كبد ذكور الجرذان الب ضاء البالغة

المشاركون في البحث

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قسم *الطب الشرعي والسموم* -ك ة الطب *الب طري* - جامعة الزقاز ق . مصر
***قسم *الطب الشرعي والسموم* الإكل ن ك ة -ك ة الطب البشرى-جامعة الزقاز ق . مصر**

الأكر لام د مركب ك م ائي شائع ح ث ستخدم في كل من الصناعة والعمل ات المعمل ة و تكون من تسخ ن الأغذ ة النشو ة وخصوصا منتجات البطاطس. الجل س دام د عتبر الأ ض النشط الذي لعب دور مركزي في التسمم الوراثي والسرطاني .
ال دف من [a]ذا البحث كان لتوض ح إمكان ة التسمم الوراثي للأكر لام د من إحداث السرطنة وتسمم الكبد بإ اس الس تكروم ب 2E1 450 والبروت ن الوراثي الحامض النووي الر بوزى الرسول في الفئران المتسممة بالأكر لام د . وتسج ل ت تك الحامض النووي الر بوزى في فصوص الكبد بواسطة اختبار كوم ت .
تم استخدام ثلاثون جرذ ناضج في [a]ذه الدراسة وتم تقس م م عشوائ ا إلى ثلاثة مجموعات .
المجموعة الأولى تم تجر ع ا بالفم ب 50 مجم / كجم لمدة 21 وم .
المجموعة الثان ة تم تجر ع ا بالفم ب 100 مجم / كجم لمدة 21 وم.

المجموعة الثالثة : مجموعة ضابطة .
وأوضحت نتائج ^aذه الدراسة أن الأكر لاه د احدث تغ ر واضح في سلوك الح وانات واحدث موت مبكر في جرذان المجموعة ن المعالجة ن وصلت نسبة إلى 31% مع المجموعة المعالجة بالجرعة الصغ رة و 38% في المجموعة المعالجة بالجرعة الكب رة . وقد احدث ارتفاع ذو دلالة إحصائ ة اقل من 5 0.0 في انز مى الترنساه ناز وانخفاض معنوي فى مستو ات البروت ن الكلى والألب وم ن والجلوب ول ن .
وقد أوضحت طر قة ق اس البروت ن المناعي الس تكروم ب 2E1 انحدار مستوى البروت ن في الس تكروم 2E1 في المجموعة المعالجة بالأكر لاه د بشكل معنوي .
أ ضا انحدار ج ن الحامض النووي الر بوزي الرسول والمعبر عن الس تكروم ب 2E1 . وقد كان ^aناك ز ادة إحصائ ة فى تحطم الشر ط المنفرد للحامض النووي الر بوزي بطر قة كوم ت .
وبالفحص النس جي لكبد المجموعات المعالجة بالأكر لاه د أوضح تغ رات تكرز ة ظ رت فى صورة حب بات زرقاء في الس توبلازم وتحلل بعض الانو ة وتغلغل الخلا ا المستد رة في الأنسجة الباب ة والخلالا ة . بجانب اتساع الشع رات في بعض ج وب الكبد وبعض التغ رات التنكس ة كانت واضحة ح ث تم ز ت بانتفاخ غ مي وت تك مائي فى بعض خلا ا الكبد مع نقص نمو في خلا ا كبفر .كل التغ رات المحدثة في جم ع الق اسات التى خضعت للدراسة كانت مرتبطة بز ادة الجرعة .
وفى الن ا ة نوصى بالحد من التعرض للاكر لاه د فى الصناعة أو المنتجات الغذائ ة بالإضافة لز ادة توع ة الناس عن أضراره.