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Original Research

Effects of alpha lipoic acid on acrylamide-induced hepatotoxicity in rats

F. A. Al-Qahtani[1], M. Arafah[2], B. Sharma[3], N. J. Siddiqi[1*]

[1] Department of Biochemistry, College of Science, King Saud University, Riyadh, Kingdom of Saudi Arabia
[2] Department of Pathology, College of Medicine, King Saud University, Riyadh, Saudi Arabia
[3] Department of Biochemistry, University of Allahabad, Allahabad 211002, India

Correspondence to: nikhat@ksu.edu.sa

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Abstract: Acrylamide (ACR) is a neurotoxicant, reproductive toxicant, and carcinogen in animal species. It is used in many industries and has been found to form naturally in foods cooked at high temperatures. Alpha-lipoic acid (ALA) is a naturally occurring antioxidant whose therapeutic effect has been related to its antioxidant activity. This study was carried out to study the protective effect of alpha lipoic acid on acrylamide induced perturbations in rat liver. Four groups of rats were studied viz., control rats, acrylamide treated rats, alpha lipoic acid treated rats, and alpha lipoic acid plus acrylamide treated rats. ACR and ALA treatment alone and together caused a significant increase in hepatic reduced glutathione content while a decrease in hepatic ascorbic content was observed when compared to control group. ALA pretreatment of acrylamide exposed rats caused no a significant alteration in superoxide dismutase activity but resulted in a tendency towards restoration of glutathione peroxidase and catalase activity to near normal levels. Gel electrophoresis showed fragmentation of DNA in the treated groups. The dose of ALA used in the present study afforded partial restoration of oxidative indices altered by ACR in rat liver.

Key words: Acrylamide; Lipoic acid; Oxidative stress; Antioxidants; Rat; Liver.

Introduction

Acrylamide (ACR) is used in many industries and has been found to form naturally in foods cooked at high temperatures. ACR has been shown to be a neurotoxicant, reproductive toxicant, and carcinogen in animal species (1). ACR causes cellular damage in the nervous and reproductive systems and produces tumors in certain hormonally responsive tissues. ACR is the source of oxidative stress in the biological systems (2). Acrylamide has been reported to increase the oxidative stress parameters, which cause disturbances in antioxidant enzymes at high doses (2). In rodents, acrylamide has been reported to increase lipid peroxidation and impair antioxidant defense system (3, 4). Extensive studies in rodents and other laboratory animals have provided evidence that exposure to ACR causes cellular damage in the nervous and reproductive systems. Yousef and El-Demerdash (5) have shown acrylamide to be neurotoxic in experimental animals and humans. Their results have showed that acrylamide causes necrosis of hepatocytes, disruption of blood sinusoid and decreased number of kupffer cells (5).

Alpha-Lipoic acid (ALA) is a naturally occurring nutraceutical. Therapeutic effect of ALA is related to its antioxidant activity and its ability to repair oxidative damage (6). ALA has been used in the prevention or treatment of several pathological conditions that are mediated by oxidative stress. Sandhya and Varalakshmi (7) reported that α-LA can prevent oxidative stress by increasing free radical scavenging capacity and inhibiting lipid peroxidation and GSH depletion. ALA has been reported to protect against oxidative damage by chelating metal ions (8). ALA has also been reported to activate phase II detoxification of xenobiotics (8) and exert hepatoprotective effects by various mechanisms (9). In this study, an attempt has been made to study the protective effect of alpha lipoic acid on acrylamide induced alterations in rat liver.

Materials and Methods

Chemicals

All the chemicals were purchased from Sigma Chemical Co. St Louis, MO, USA.

Animals

Healthy adult male Wistar rats weighting 200-250g (four to six week old) were obtained from the animal house of King Saud University, Riyadh, Saudi Arabia. The animals were labeled by identifying ear notches, housed in clean cages and placed in the animal care room. Rats were allowed free access to food (Purina rodent chow) and tap water. Ethical animal care guidelines were followed and the study was approved by the animal ethics committee of College of Science, King Saud University, Riyadh, Saudi Arabia (Approval number - 4/67/112564).

Treatment

Animals were divided into 4 groups. Each group consisted of 10 animals. The experimental groups were Group 1: Control rats, Group 2: Acrylamide group rats injected with 50 mg/kg body weight acrylamide, intra-

peritoneally for four weeks, Group 3: Alpha lipoic acid group- Rats injected with 35 mg/kg body weight lipoic acid, intraperitoneally for four weeks, Group 4: Alpha lipoic acid and acrylamide group - Rats injected with lipoic acid at dose of 35 mg/kg body weight followed by acrylamide 50 mg/kg body weight after one hour for four weeks.

Preparation of sample

The animals were killed 24 hours after the last dose of injected compound by CO_2 asphyxiation. The liver was dissected out, cleaned of the adhering tissues, weighed and homogenized in normal saline (10% W/V). The homogenate was centrifuged at 3000g for 10 min. The resulting supernatant was used for biochemical assays.

Biochemical assays

Reduced glutathione was determined by the method of Beutler et al., 1963 (10).

Ascorbic acid was determined by the method of Jagota and Dani, 1982 (11).

Glutathione peroxidase was assayed using the commercial kit purchased from Cayman, USA following manufactures instruction.

The activity of glutathione reductase was determined by the method of Goldberg and Spooner, 1987 (12).

Superoxide dismutase was assayed using commercial kit purchased from BioVision, USA following manufactures instructions.

Catalase activity was estimated in the whole homogenate by the method of Aebie H, 1984 (13).

Protein content was determined in the whole tissue homogenate by the modified method of Markwell et al. 1978 (14).

DNA was isolated from liver tissue and electrophoresis was carried out according to the method of Iwasa et al. 1996 (15).

Preparation of the Sample for Light Microscopy

The liver was removed and fixed in 10% formalin and stored till further processed for microscopy. Tissue were processed to obtain 5µm thick paraffin wax, stained with haematoxylin and eosin according to Bancroft and Stevens, 1996 (16) and used for histopathological examination.

Statistical analysis

Values were expressed as mean ±S.D. The data were statistically representative in terms of number, mean and standard deviation (S.D.). Comparison between control group and each other group was done using Independent Samples T-Test for Parametric Parameters and Mann-Whitney for None-ParametricParameters and Comparison between all groups using One-Way ANOVA for Parametric Parameters and Kruskal-Wallis for None-Parametric Parameters. P value less than 0.05 was considered statistically significant.

Results

The results of the effect of acrylamide and lipoic acid treatment individually and in combination on reduced glutathione content in rat liver are shown in Figure 1. The data indicated that acrylamide treatment caused a significant increase (199%) in reduced glutathione content when compared to control (P<0.001). Lipoic acid also caused a significant increase (266%) in reduced glutathione content when compared to control (p<0.001). Treatment of lipoic acid and acrylamide together caused a significant increase in reduced glutathione level by 215% when compared to control (P<0.001).

The effect of acrylamide and lipoic acid treatment alone and in combination on ascorbic acid levels in rat liver was observed and the results are shown in Figure 2. It was found that acrylamide treatment caused a significant decrease (66%) in ascorbic acid concentration

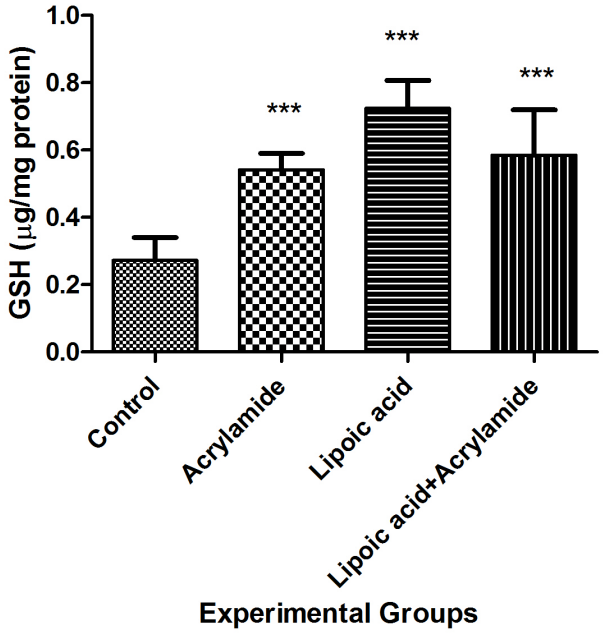

Figure 1. Effect of acrylamide and lipoic acid treatment on reduced glutathione content in rat liver. ***P < 0.001 when compared to control group.

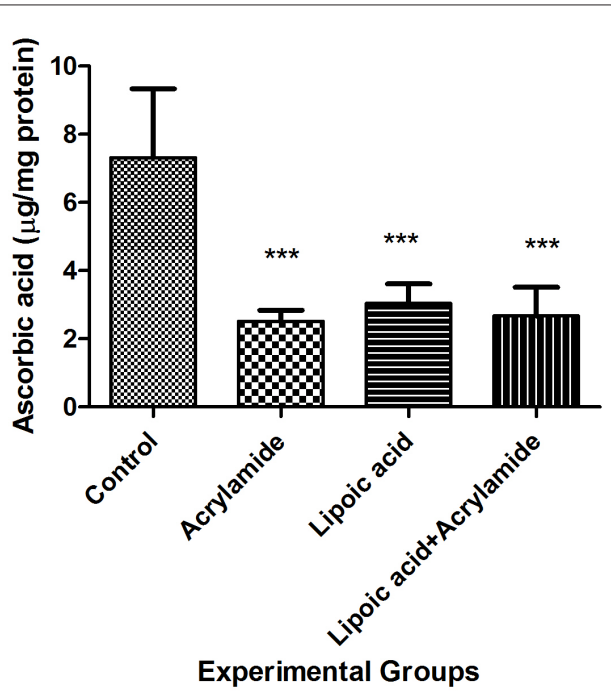

Figure 2. Effect of acrylamide and lipoic acid treatment on ascorbic acid levels in rat liver. ***P < 0.001 when compared to control group.

of rat liver when compared to control liver (P<0.001). The lipoic acid treatment also caused a significant decrease (58%) in ascorbic acid level when compared to control (p<0.001). However, the combined treatment of lipoic acid and acrylamide did not restore ascorbic acid concentration to normal value.

Figure 3 shows the effect of acrylamide and lipoic acid treatment alone and in combination on glutathione peroxidase (GPx) activities. The results demonstrated that acrylamide and lipoic acid caused a significant increase (1087%) and (6318%) respectively in glutathione peroxidase activity when compared to control (P<0.01). Treatment of lipoic acid and acrylamide together caused no any significant increase in glutathione peroxidase activity when compared to control, there by showing a tendency toward restoration of GPx activity.

The results of the effects of acrylamide and lipoic acid treatment alone and in combination on glutathione reductase (GR) activity are shown in Figure 4. Acrylamide and lipoic acid treatment caused no any significant change in GR activity when compared to control. The combined treatment of lipoic acid and acrylamide caused a significant decrease in GR activity by 37% when compared to control.

The effects of acrylamide and lipoic acid treatment alone and in combination on superoxide dismutase (SOD) activities are shown in Figure 5. Acrylamide and lipoic acid alone and in combination caused no any significant alteration (P>0.05) in SOD activity of rats liver.

Like the GST and the SOD, the catalase is also an an-

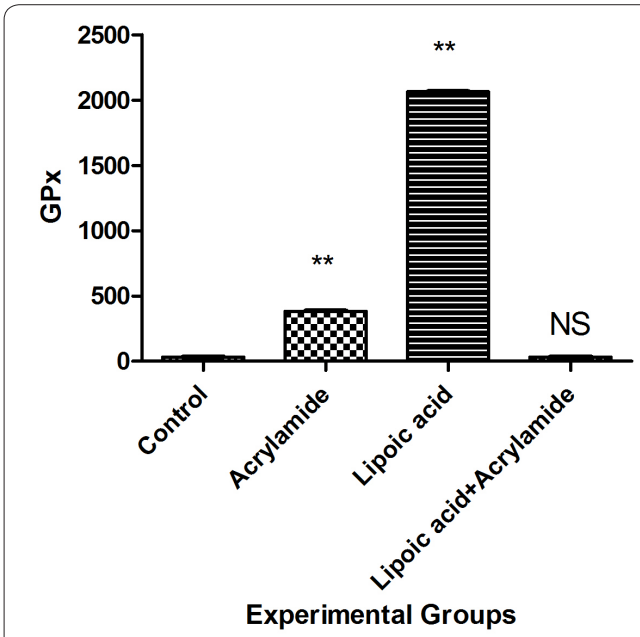

Figure 3. Effect of acrylamide and lipoic acid treatment on glutathione peroxidase (GPx) activity in rat liver. **P < 0.01 when compared to control group.

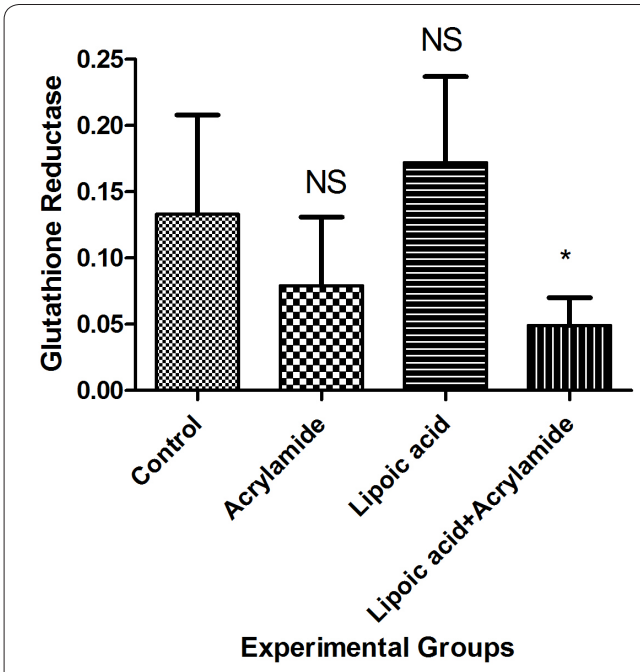

Figure 4. Effect of acrylamide and lipoic acid treatment on glutathione reductase activity in rat liver. *P < 0.05 when compared to control group. NS not significant when compared to control group.

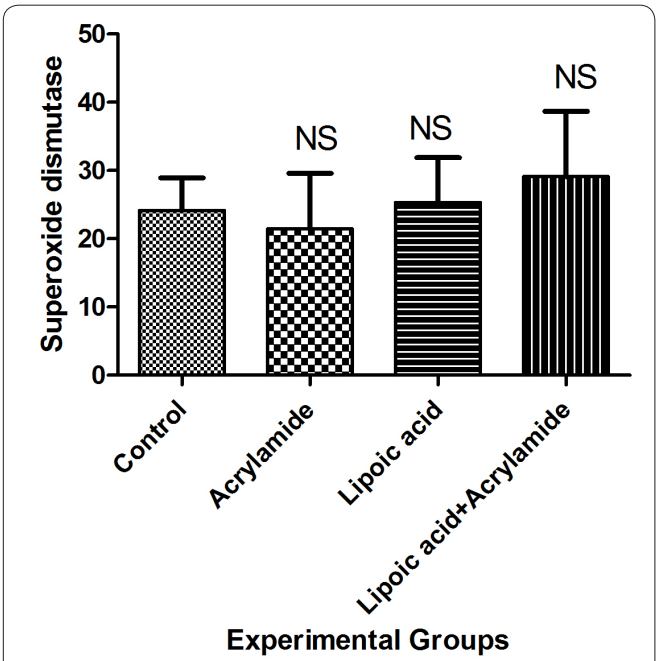

Figure 5. Effect of acrylamide and lipoic acid treatment on superoxide dismutase activities in rat liver. NS not significant when compared to control group.

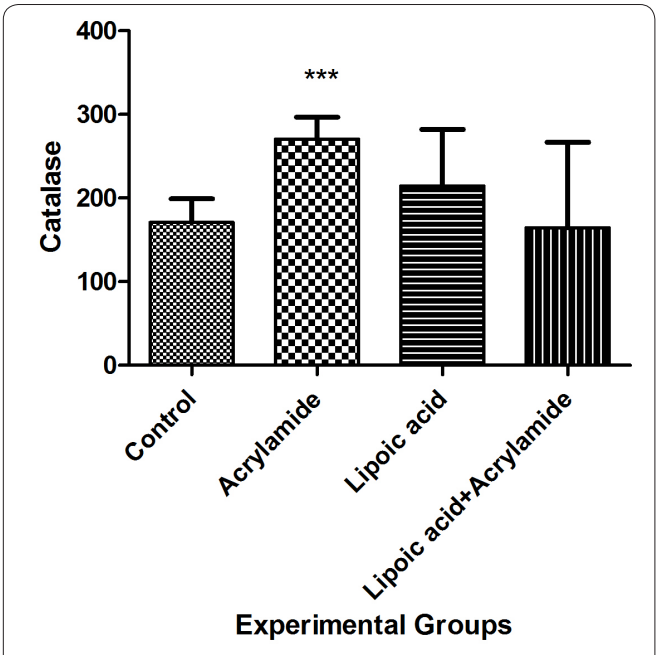

Figure 6. Effect of acrylamide and lipoic acid treatment on catalase activity in rat liver. ***P < 0.001 when compared to control group.

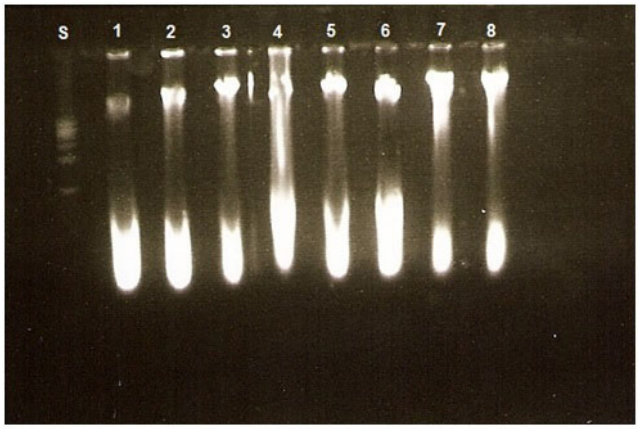

Figure 7. Effect of acrylamide and lipoic acid treatment alone and in combination on rat liver DNA. S: Molecular weight standard. Lane 1-2: control rats, lane3-4: acrylamide treated rats, lane5-6: alpha lipoic acid treated rats, lane7-8: alpha lipoic acid plus acrylamide treated rats.

tioxidant enzyme. Hence the effects of acrylamide and lipoic acid treatment on catalase activity were observed in different groups of rats and the results are shown in Figure 6. The data indicated that the acrylamide treatment caused a significant increase in catalase activity by 158% when compared to control ($P<0.001$). The lipoic acid treated rats showed no significant alteration in catalase activity when compared to control rat. Treatment of lipoic acid and acrylamide together restored the altered catalase activity to near normal level.

The DNA being the genetic material may also interact with the xenobiotics such as acrylamide. The evaluation of the impacts of acrylamide and lipoic acid treatment alone and in combination on DNA in different groups of rats was observed as shown in Figure 7. The results indicated that acrylamide treatment caused fragmentation of DNA. In lipoic acid treated rats no significant fragmentation was observed. The combined treatment of lipoic acid plus acrylamide caused slight fragmentation of DNA (Figure 7).

The effects of acrylamide and lipoic acid were also evaluated on the rat's histological parameters. Figures 8(a), 8(b), 8(c) and 8(d) show the sections of the livers of a control rat, acrylamide treated, alpha lipoic acid treated rat and alpha lipoic acid plus acrylamide treated rats, respectively. Acrylamide treated rat liver showed sinusoidal congestion in central vein, blood vessels with obvious swelling of the hepatocytes, mononuclear inflammatory cells and hydropic degeneration. The liver of rat treated with alpha lipoic acid group showed mild mononuclear inflammatory cells.

Discussion

Acrylamide is a low molecular weight industrial chemical used in the production of polyacrylamides for water and waste water treatment, crude oil, mineral, concrete, textile, paper and pulp processing, soil and sand treatment, cosmetics and coating applications (17). Acrylamide is also found in many foods during processes like frying, baking or roasting, as a result of Maillard reactions involving the amino acid asparagine and reducing sugars. The biological consequences of acrylamide exposure have mainly centered on neurotoxicity ever since this effect was observed in humans occupationally exposed to this compound (18). The liver is a one of the target organ of toxins. It is centrally located between the gastrointestinal tract where absorption of ingested drugs occurs and is central to the metabolism of nearly all xenobiotics. Biochemical processes in the hepatocyte metabolize many compounds to make them hydrophilic, resulting in metabolites that are water soluble and can be excreted in the bile or urine (19).

In this study acrylamide caused a significant increase in hepatic lipid peroxidation (unpublished data) as indicated by an increase in MDA levels in livers of ACR exposed rats when compared to control rats. Acrylamide has been reported to increase lipid peroxidation by inducing oxidative stress with generation of free radicals (20). These results are in agreement with Srivastava et al. 1983 (21) who showed an increase in lipid peroxidation in brain and liver of rats upon acrylamide administration. Cellular fatty acids are readily oxidized by ROS to produce lipid peroxyl radicals and lipid hydroperoxides (22). Lipid peroxyl radicals can subsequently propagate into malondialdehyde (MDA) and these results have been attributed the significant increased lipid peroxidation in acrylamide treated rats (23). MDA levels in liver may also be used to investigate the oxidative damage of protein and lipoproteins, which is a possible pathogenic mechanism for liver injury (24).

Acrylamide treatment caused a significant increase in hepatic reduced glutathione when compared to control rats. Studies of Beiswanger et al. (25) have shown that acrylamide decreases the reduced glutathione content in the liver and brain possibly due to its detoxification by conjugation with reduced glutathione. However, in the present study an increase in hepatic GSH concentration was observed following ACR treatment. This may be

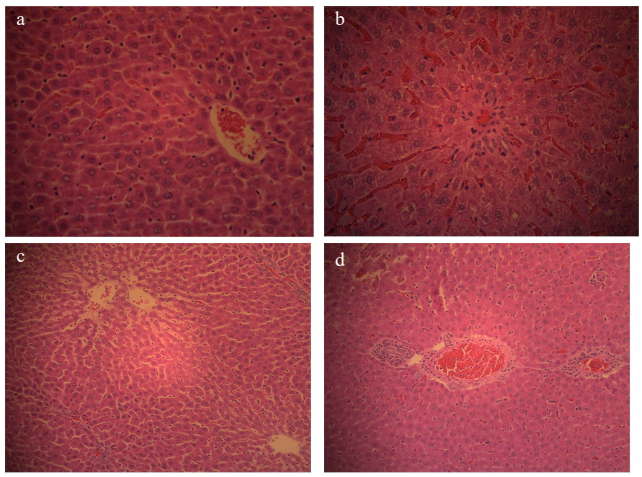

Figure 8. (**a**): A photomicrograph of a section of the liver of a control rat; showing normal in histological appearance (H&E., x 40). (**b**): A photomicrograph of a section of the liver of acrylamide treated group showing sinusoidal congestion in central vein (CV), blood vessels with obvious swelling of the hepatocytes, mononuclear inflammatory cells and hydropic degeneration (arrow) (H&E., x 40). (**c**): A photomicrograph of a section of the liver of alpha lipoic acid treated group. This group showed mononuclear inflammatory cells (H&E., x 20). (**d**): A photomicrograph of a section of the liver of alpha lipoic acid plus acrylamide treated group. This group showed mild congestion of portal vein (PV), mild mononuclear inflammatory cells and hydropic degeneration(arrow) (H&E., x 40

attributed to the initial response of the liver to acrylamide assault or a lower dose of ACR used in the present study. At a low dose, the initial response to the ACR assault may be to increase the hepatic GSH levels to cause an increased detoxification of ACR (26). α-Lipoic acid has been reported to possess the ability to correct deficient thiol status of cells (27), increase de novo synthesis of GSH (28) and enhance GSH levels in diabetic and normal animals (29). Inside cells and tissues, alpha lipoic acid is reduced to dihydrolipoic acid, which is even more potent as an antioxidant (30). Glutathione is an important antioxidant that is synthesized from the sulfur-containing amino acid cysteine. Lipoic acid induces cysteine uptake thereby increasing synthesis of glutathione both in vitro and in vivo. Han et al. (28) proposed that dihydrolipoic acid reduced cystine to cysteine, which is the limiting substrate for GSH synthesis. Additionally, LA may also increase cellular cysteine levels by enhancing cystine uptake from plasma followed by its reduction to cysteine (28). Combined treatment of ALA and ACR resulted in increased hepatic GSH levels when compared to control probably due to the predominant effect of ALA.

Acrylamide treatment caused a significant decrease in ascorbic acid concentration when compared to control rats. LA also caused a significant decrease in hepatic ascorbic acid concentration when compared to control rats. ACR has been shown to deplete ascorbic acid levels in the brain of chick embryo (31). Combined treatment of LA and ACR did not restore ascorbic acid to normal levels in rat liver.

Lebda et al. (32) have reported a significant decrease in the activities of glutathione peroxidase (GPx) and glutathione reductase (GR) in the testes of ACR treated rats. In the present study acrylamide and LA treatment individually to rats caused no significant alterations in the activities of GR which may be due to a shorter duration of the study or lower doses used. Combined treatment of LA and ACR caused a significant decrease in GR activity. The decrease in GR activity may be due to its inhibition by GSH which was concomitantly increased in the same group. The increase in glutathione peroxidase activity after treatment with LA, is most readily explained as a necessary consequence of inhibiting formation of free radicals. Glutathione peroxidase catalyzes the reduction of hydrogen peroxide to water and oxygen at the expense of glutathione (GSH). Therefore, the increase in glutathione peroxidase activity indicates that more of oxidized glutathione (GSSH) is reduced to GSH. Combined treatment of rats with ACR and lipoic acid resulted in restoration of GPx activities to near normal levels.

In the present study, lipoic acid alone and with acrylamide caused no significant changes in hepatic catalase and superoxide dismutase activities. Acrylamide treatment of rats resulted in a significant increase in hepatic catalase activity. Although ACR treatment has been reported to decrease hepatic catalase activity (33), the opposite effect observed in the present study may be attributed to a shorter duration of ACR treatment or the immediate response of the body to initial acrylamide assault.

The carcinogenicity of acrylamide has been attributed to its metabolite glycidamide which reacts with DNA to give a number of DNA adducts (17). In the present study DNA fragmentation was observed in ACR treated rats as demonstrated by agarose gel electrophoresis. In LA treated rats, no significant fragmentation was observed. However, in LA plus ACR treated groups also slight fragmentation of DNA was observed. Acrylamide has been reported to cause only weak and transient DNA damage was recorded in the liver (34). These results may demonstrate that LA does not prevent the metabolism of ACR by cytochrome 450 to its metabolite glycidamide.

ACR treated rats showed sinusoidal congestion in central vein while hepatocytes of the lipoic acid treated group displayed mild mononuclear inflammatory cells which correlates with the increased lipid peroxidation observed in these groups and may explain the biochemical effects of this study.

Thus, at the dose of LA used in the present study there was only a partial restoration of indices altered by ACR in rat liver. Studies on diabetic neuropathy have indicated that beneficial effects of LA may be observed only at a relatively high dose, i.e., 600 mg/d (35). Lack of significant effects may also be due to rapid metabolism and the excretion of the metabolites in urine (8).

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