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Azelaic Acid Attenuates Ethanol-Induced Hepatotoxicity in Experimental Rats

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Abstract

Alcohol abuse or prolonged consumption of alcohol leads to alcoholic liver disease. Alcoholic liver disease is the spectrum of liver injury starting from steatosis to liver cancer. The goal of this study was to investigate the effect of azelaic acid on ethanol induced liver toxicity in experimental rats. Materials and methods: Rats were divided into six groups. Group 1 rats received isocaloric glucose. Group 2 rats received isocaloric glucose and azelaic acid (80 mg/kg b.w). Group 3 rats received ethanol alone (5g/kg b.w). Group 4-6 rats received ethanol and different doses of azelaic acid (20, 40 or 80 mg/kg.b.w). Key findings: The ethanol treated rats showed significantly elevated activities of liver marker enzymes such as AST, ALT, ALP, GGT. The lipid peroxidation markers such as lipid hydroperoxides, conjugated dienes and thiobarbituric acid reactive substances were significantly elevated as compared to the control. The liver alcohol metabolizing enzyme such as the activity alcohol dehydrogenase activity was elevated while the activity of aldehyde dehydrogenase was reduced. In addition, the antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, reduced glutathione, vitamin C and vitamin E showed decreased activities/levels. Azelaic acid supplementation reduced the levels of ethanol-induced lipid peroxidation, optimized the antioxidant status, increased the activity of aldehyde dehydrogenase enzyme and cleared the blood acetaldehyde levels in the serum. Conclusion: This study reveals that azelaic acid protects the liver against ethanol induced toxicity.

Keywords

Ethanol; Azelaic acid; Lipid peroxidation, Alcoholic liver disease, Antioxidants.

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INTRODUCTION

Alcohol is one of the leading causes of end-stage liver disease throughout the world (1). Alcoholic liver disease generally begins with asymptomatic steatosis, but can stealthily progress to steatohepatitis, fibrosis irreversible cirrhosis and hepatocellular carcinoma (HCC) (2). Experimental models show that alcohol is a true hepatotoxin, that causes hepatocellular damage (3). *In vivo* animal studies have shown that ethanol alters the morphological changes and functions of hepatocytes (4).

Oxidative stress is due to by an impaired balance between reactive oxygen species (ROS) production and removal (5). Chronic alcohol consumption leads to increased oxidative stress, cell membrane

permeability, cell necrosis and mitochondrial leakage of liver marker enzymes into the blood (6). The metabolism of ethanol generates acetaldehyde and reactive oxygen species (ROS), which may damage the macromolecules of the cell (7).

Azelaic acid is a saturated dicarboxylic acid which is widely present in grains such as wheat, rye, barley, oat seeds and sorghum. It has been reported to show antiproliferative (8), antidiabetic (9) and antileukemic properties (10). However, till date there is no report showing the modulatory effect of azelaic acid on alcohol induced liver injury. Therefore, our present study was formulated to investigate the protective effect of azelaic acid against alcohol induced hepatotoxicity.

MATERIALS AND METHODS

Chemicals and reagents

Chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol was obtained from E.I.D Parry India Ltd., (Nellikuppam, Cuddalore District, India). All other chemicals and reagents used were of analytical grade obtained from Himedia Laboratory Ltd., Mumbai, India.

Animals

Male albino Wistar rats weighing about 150-180 g were obtained from Biogen Bangalore. Rats were maintained as per the principles and guidelines of the ethical committee for animal care and use of Annamalai university in accordance with the Indian National Law on Animal Care (Reg. No. 160/1999/CPCSEA/1129).The experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Rats". The animals were housed in plastic cages with paddy husk for bedding at a temperature of 27 ± 2°C with 12h light: dark cycles.

Preparation of azelaic acid and ethanol

Azelaic acid was dissolved in warm water (60º C) cooled and then administered orally to the experimental rats. The dose was of azelaic acid fixed based on the previous literature (9). Similarly, the dose of ethanol was fixed based on the previous literature (11)

Experimental protocol

Animals were divided into six groups of six animals each. Total experimental period was 60 days.

• Group 1: Rats received standard pellet diet, and isocaloric glucose (40% glucose in drinking water) for the entire experimental period of 60 days

• Group 2: Rats received standard pellet diet along with isocaloric glucose everyday throughout the experimental period and azelaic acid (80 mg/kg b.w. p. o) was supplemented from the 31st day till the end of the experiment

• Group 3: Rats received 20% ethanol (equivalent to 5g/kgb.w.p.o) everyday throughout the experimental period of 60 days

• Group 4-6: Rats received 20% ethanol everyday throughout the experiment and azelaic acids (20, 40 or 80mg/kgb.w.p.o respectively) were supplemented from the 31st day till the end of the experiment.

At the end of experimental period, the animals were treated with ketamine hydrochloride and sacrificed. Liver and kidney were immediately cleaned with ice-cold saline (0.9 % sodium chloride), homogenized and the supernatant was used for the biochemical estimations and histological studies.

Biochemical estimations

Estimation of liver marker enzymes

The activities of serum aspartate aminotransferase (AST; E.C.2.6.1.1) and alanine aminotransferase (ALT; E.C.2.6.1.2) were estimated by the method of (12). Serum alkaline phosphatase (ALP; E.C 3.1.3.1) was assayed using the diagnostic kit based on (13) method. The serum γ-glutamyl transpeptidase (GGT; E.C.2.3.2.2) was assayed according to the method of (14). Total proteins by the method of (15) and serum albumin was estimated by the method of (16). Concentration of serum globulin was calculated using the formula: globulins = total proteins – albumin.

Alcohol dehydrogenase (ADH; E.C.1.1.1.1) and Aldehyde dehydrogenase (ALDH; E.C.1.2.1.3) were assayed by the method of (17).

Estimation of lipid peroxidation byproducts (TBARS, LOOH, CD)

Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) using the method of (18). The pink chromogen produced by the reaction of secondary products of lipid peroxidation such as malondialdehyde with thiobarbituric acid was estimated at 532 nm. The concentration of serum lipid hydroperoxides (LOOH) was estimated by the method of (19). Conjugated dienes were estimated by the method of (20). The method is based on the arrangements of double bonds in polyunsaturated fatty acids to form conjugated dienes with an absorbance maximum at 233 nm.

Assay of enzymic antioxidants

Superoxide dismutase (SOD; EC 1.15.1.1) was assayed by the method of (21). The assay was based on the 50% inhibition of the formation of NADH-phenazine methosulphate-nitroblue tetrazolium (NBT) formazan at 520 nm. The activity of catalase (CAT; EC 1.11.1.6) was estimated by the method of

(22) by monitoring the decomposition of H_2O_2 measured at 590 nm.

Glutathione peroxidase (GPx; EC 1.11.1.9) activity was determined using the method of (23). A known amount of the enzyme preparation was incubated with H_2O_2 in the presence of glutathione for a specific time period. The amount of H_2O_2 utilised was determined by the method of (24). Glutathione reductase (GR; EC 1.6.4.2) activity was determined by the method of (25). One unit of enzyme is defined as µmol NADPH consumed /min per mg protein.

Glutathione S-transferase (GST; EC 2.5.1.1.8) was assayed by the method of [26]. The change in absorbance was recorded at 340 nm and enzyme activity was calculated as µmol of 1-chloro-2, 4-dinitro benzene (CDNB) conjugate formed/min/mg protein using a molar extinction coefficient of $9.6X10^3$ min/cm.

Estimation of non-enzymic antioxidant

Reduced glutathione (GSH) was measured by the method of (24). The concentration of GSH was measured using the 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB) glutathione disulfide reductase recycling assay for GSH based on the method of (27). A known amount of enzyme preparation was incubated with H_2O_2 in the presence of GSH for a period of 5 min. The amount of H_2O_2 utilized was determined using the method of (24). Values are expressed as µmol of GSH utilized/min/mg protein.

Vitamin E was estimated by the method of (28). The method involves the α-tocopherol mediated reduction of ferric ions and the formation of a red colored complex with 2, 2'dipyridyl. The absorbance of the chromophore was measured at 520 nm. Vitamin C level was estimated by the method of (29), in which dehydro ascorbic acid is coupled and then treated with sulfuric acid, forming an orange red colored compound, which was measured at 520 nm.

STATISTICAL ANALYSIS

Results were expressed as means ± SD of six rats per group. Data were analysed by one-way analysis of variance (ANOVA) and any significant differences among the treatment groups were evaluated using Duncan's multiple range test (DMRT). Results were considered statistically significant when $P< 0.05$. All statistical analyses were performed using SPSS version 15.0 software package (SPSS, Tokyo, Japan).

RESULTS

Table 1 shows the activities of the liver marker enzymes such as AST, ALT, ALP, and GGT of the control and ethanol treated experimental rats. Rats treated with azelaic acid alone (Group 2) did not show any statistically significant ($P < 0.05$) difference in the activities of liver marker enzymes as compared to that of the control rats. Ethanol treated rats (Group 3) showed significantly elevated activities of AST, ALT, ALP, and GGT as compared to the control groups, whereas on supplementation with azelaic acid (Group 4-6) to ethanol fed rats, the activities of these enzymes were significantly decreased ($P < 0.05$) as compared to the ethanol alone fed rats.

Figure 1: shows the activities of serum ADH and ALDH in the liver of the control and experimental rats. Rats treated with azelaic acid alone (Group 2) did not show any statistically significant ($P < 0.05$) difference in the activities of the alcohol metabolizing enzymes as compared to that of the control rats. Ethanol treated rats (Group 3) showed a significant ($P < 0.05$) increase in the activity of ADH and a significant decrease in the ALDH activity (Group III) as compared to those of the control rats. Azelaic acid co-treatment (Groups 4-6) to ethanol fed rats, showed a significant ($P < 0.05$) decrease in ADH and an increase in the ALDH activities as compared to the ethanol alone fed rats.

Table 2 shows the levels of serum and hepatic lipid peroxidative byproducts of control and experimental rats. Treatment with azelaic acid to control rats (Group 2) did not show any significant changes in the levels of TBARS, LOOH and CD. Ethanol treated rats (Group 3) showed significantly increased lipid peroxidation byproducts as compared to the control rats (Group 1). Azelaic acid co-treatment to ethanol administered rats (Group 4-6) $P < 0.05$ significantly decreased the levels of TBARS, LOOH and CD levels when compared to the un-supplemented ethanol treated rats (Group 3).

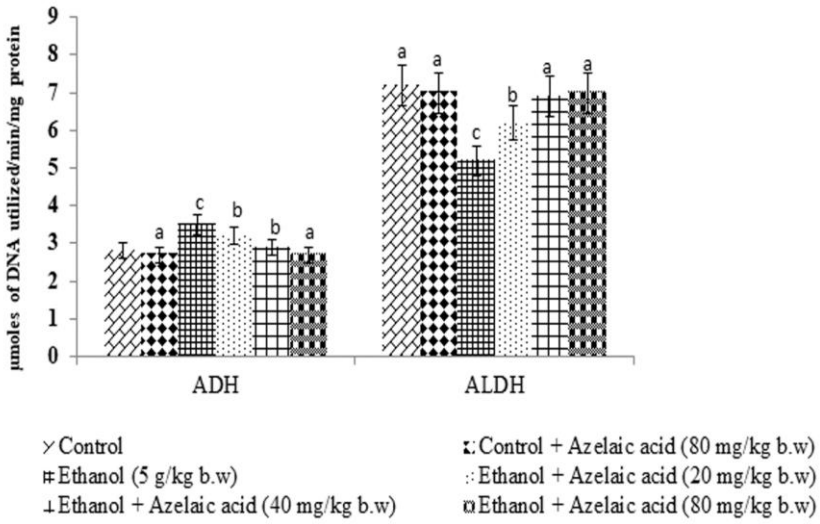

Figure 1: Effect of azelaic acid and ethanol on hepatic alcohol metabolizing enzymes of control and experimental rats. Values are given as mean ± standard deviation for six rats in each group. Groups not sharing a common superscript letter differ significantly at P < 0.05. DMRT = Duncan's multiple range test; ADH = Alcohol dehydrogenase; ALDH = Aldehyde dehydrogenase.

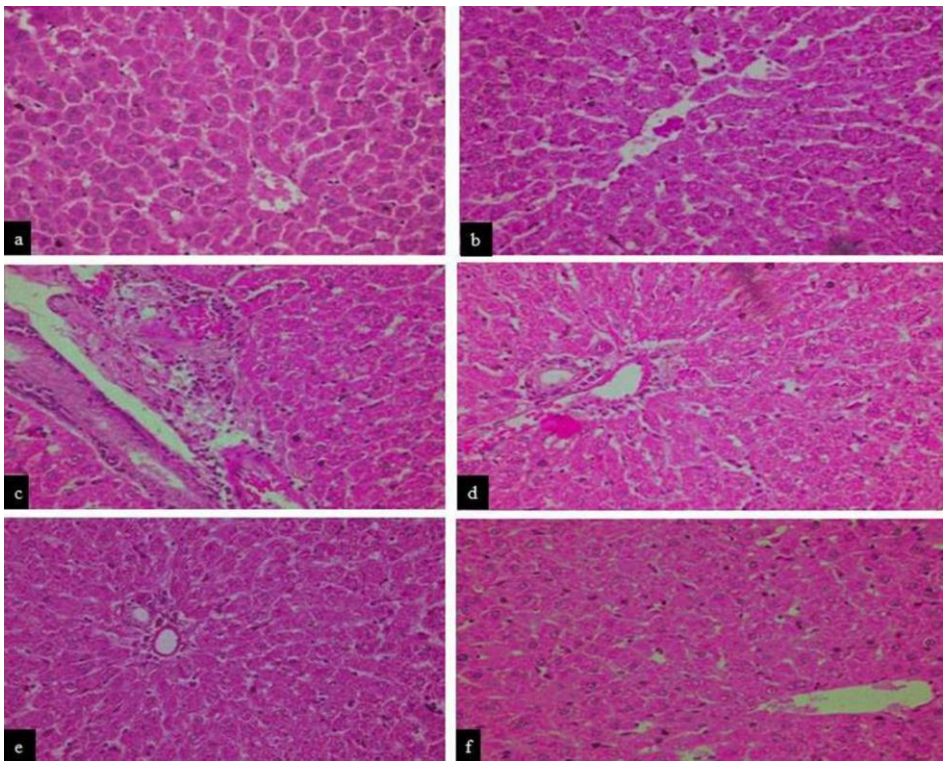

Figure 2: Liver histology of control and experimental rats (H and E, ×40). (a)The liver of control rats shows the central vein and hepatocytes arranged in the form of cords. (b) Azelaic acid alone treated rat liver shows well dilated sinusoids with the hepatocytes closest to the central vein, and portal triad appears normal. (c) Ethanol administered rat liver shows hepatocytes with fatty infiltration and inflammatory cells, micro and macrovesicular fatty changes, periportal fibrosis and vascular congestion. (d) Ethanol + azelaic acid (20 mg/kg b.w) treated rat liver shows reduced cytoplasmic vacuolation, sinusoidal dilation and centrilobular necrosis.(e) Ethanol + azelaic acid (40 mg/kg b.w) shows reduced sinusoidal dilation and periportal fibrosis. (f) Azelaic acid (80 mg/kg b.w) alone supplementation shows near normal hepatic architecture.

Table 1 Effect of azelaic acid and ethanol on the activity of hepatic marker enzymes in the serum of the control and experimental rats.

Groups	Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)
I	Control	78.49 ± 5.98^{a}	29.12±2.23^{a}	98.32± 7.49^{a}	11.82 ± 0.90^{a}
II	Control +Azelaic acid (80 mg/kg b.w)	76.82 ± 5.88^{a}	29.70±2.26^{a}	97.65 ± 7.48^{a}	9.80 ± 0.75^{a}
III	Ethanol (5 g/kg b.w)	116.12± 8.84^{d}	66.10±5.06^{d}	169. 10 ±12.88^{d}	27.52± 2.11^{b}
IV	Ethanol +Azelaic acid (20 mg/kg b.w).	102.22 ±7.83^{b}	55.25±4.21^{c}	141. 20±10.75^{c}	18.62±1.43^{d}
V	Ethanol +Azelaic acid (40 mg/kg b.w).	76.92± 5.86^{c}	29.30±2.23^{b}	101.22±7.71^{b}	12.35±0.95^{b}
VI	Ethanol +Azelaic acid (80 mg/kg b.w).	88.20±6.75^{a}	34.13±2.60^{a}	114.52±8.72^{a}	15.20±1.16^{a}

Values are given as mean ± SD of each group. Superscript letters ($^{a–d}$) are used to refer and distinguish the values of the different groups. Values not sharing a common superscript differ significantly at p<0.05 (DMRT).

Table 2 Effect of azelaic acid on levels of thiobarbituric acid reactive substances, lipid hydroperoxides, conjugated dienes.

Groups	Treatment	TBARS♥	LOOH♣	CD♠
I	Control	0.71±0.05^{a}	61.30±4.64^{a}	52.04±3.96^{a}
II	Control + Azelaic acid (80 mg/kg b.w)	0.73±0.06^{a}	61.79±4.73^{a}	49.59±3.80^{a}
III	Ethanol (5 g/kg b.w)	1.92±0.15^{c}	85. 09 ±6.48^{d}	84.43±6.43^{d}
IV	Ethanol +Azelaic acid (20 mg/kg b.w)	1.80±0.14^{b}	78.58±6.01^{c}	69.09±5.26^{c}
V	Ethanol +Azelaic acid (40 mg/kg b.w)	1.16±0.09^{b}	70.71±5.42^{b}	62.50±4.79^{b}
VI	Ethanol +Azelaic acid (80 mg/kg b.w)	0.76±0.06^{a}	63.09±4.80^{a}	55.01±4.19^{a}

♥ TBARS nmol/ml, ♣LOOH mmol/ml, ♠CD mmol/ml.

Values are given as mean ± SD of each group. Superscript letters ($^{a–c}$) are used to refer and distinguish the values of the different groups. Values not sharing a common superscript differ significantly at p<0.05 (DMRT).

Table 3: Effect of azelaic acid on liver enzymic antioxidants in the control and experimental rats.

Groups	Treatment	SOD♥	CAT♦	GPx♠	GR•	GST▲
I	Control	7.89±0.60^{a}	81. 32±6.19^{a}	14.00±1.07^{a}	24.06±1.83^{a}	8.51±0.65^{a}
II	Control + Azelaic acid (80 mg/kg b.w)	8.01±0.61^{a}	81.38±6.2^{a}	14.20±1.08^{a}	24.01±1.84^{a}	8.45± 0.64^{a}
III	Ethanol (5 g/kg b.w)	3.02±0.23^{c}	56.52±4.30^{c}	6.62±0.51^{c}	11.69±0.90^{c}	4.75±0.36^{c}
IV	Ethanol +Azelaic acid (20 mg/kg b.w).	6.16±0.47^{b}	58.92±4.51^{b}	10.31±0.79^{b}	15.73±1.20^{b}	4.01±0.31^{b}
V	Ethanol +Azelaic acid (40 mg/kg b.w).	6.32±0.48^{b}	65.82±5.01^{b}	12.57±0.96^{b}	18.87±1.45^{b}	8.25± 0.63^{b}
VI	Ethanol +Azelaic acid (80 mg/kg b.w).	7.19±0.55^{a}	72.64±5.53^{a}	13.85±1.06^{a}	22.07±1.68^{a}	8.30±0.64^{a}

♥ SOD 50% NBT reduced/min/mg protein; ♦ CAT µmol of H_2O_2 utilized/min/mg protein; ♣ GPx µmol/mg protein; • GR µmol of NADPH oxidized/min/mg protein; ▲GST µmoles of CDNB-GSH conjugate formed/min/mg protein. Values are given as mean ± SD of each group. Superscript letters ($^{a–c}$) are used to refer and distinguish the values of the different groups. Values not sharing a common superscript differ significantly at p<0.05 (DMRT).

Table 4: Effect of azelaic acid on liver non enzymic antioxidants in the control and experimental rats.

Groups	Treatment	GSH ♥	Vitamin C ♦	Vitamin E ▲
I	Control	17.62± 1.3[a]	4.8±0.37[a]	1.20± 0.09[a]
II	Control +Azelaic acid (80 mg/kg b.w)	17.86±1.36[a]	5.0±0.38[a]	1.23± 0.09[a]
III	Ethanol (5 g/kg b.w)	11.80±0.90[c]	3.05±0.23[c]	7.36± 0.03[c]
IV	Ethanol +Azelaic acid (20 mg/kg b.w)	13.75±1.05[b]	3.83±0.23[b]	8.70± 0.67[c]
V	Ethanol +Azelaic acid (40 mg/kg b.w)	15.63±1.19[b]	4.2± 0.32[b]	9.07± 0.69[b]
VI	Ethanol +Azelaic acid (80 mg/kg b.w)	16.17±1.24[a]	4.7± 0.36[a]	9.75 ± 0.74[a]

♥ GSH mmol/mg protein; ♦ Vitamin C mg/dl; ▲ Vitamin E mg/dl. Values are given as mean ± SD of each group. Superscript letters (a–c) are used to refer and distinguish the values of the different groups. Values not sharing a common superscript differ significantly at $p<0.05$ (DMRT).

Table 3 shows the activities of enzyme antioxidants such as SOD, CAT, GPx, GR and GST, in the liver of control and ethanol treated rats. Group 2 rats did not show any significant changes in the levels of antioxidant enzymes, as compared to the control. The ethanol treated rats (Group 3), showed a significant ($P < 0.05$) decrease in the activities of the above enzymes as compared to the control rats (Group 1). Azelaic acid supplemented, ethanol fed rats (Groups 4-6) exhibited a significant ($P < 0.05$) increase in the activities of liver antioxidant enzymes, however the effect was more pronounced in the rats co-treated with 80 mg/kg body weight azelaic acid (Group 6) as compared to the other two doses.

Table 4 depicts the effect of azelaic acid on ethanol induced control and experimental rats of hepatic non-enzymic antioxidants such as GSH, Vitamin C and E levels of control and experimental rats. The levels of non-enzymic antioxidants were found to be significant ($P < 0.05$) decreased in ethanol treated rats (Group 3) as compared to that of the control rats (group I). Ethanol administered rats on co-treatment with azelaic acid (Group 4-6) showed a significant ($P < 0.05$) increase in non-enzymic antioxidants as compared to that of ethanol alone fed rats (group III) the effect was more pronounced in the rats supplemented with 80 mg/kg body weight azelaic acid (Group 6).

Figures 2 shows the histological changes in the liver of control and experimental rats. Liver of the control rat shows normal structure with the normal appearance of the hepatocytes and central vein (Group 1). The levels of azelaic acid administered control rat portray hepatocytes with normal lobular architecture (Group 2). Ethanol fed rat liver (Group 3) shows variations in the hepatocytes such as impaired filtration with inflammatory cells, micro- and macro-vesicular fatty changes and eroding of the portal triad and lobules. Supplementation with azelaic acid (20, 40, or 80 mg/kg b.w) to ethanol-fed rats effectively reduced the pathological abnormalities.

DISCUSSION

Alcohol abuse is a serious health problem worldwide. Liver is the major metabolic site for ethanol detoxification. Excessive intake of alcohol leads to ROS generation culminating in alcoholic liver disease (30). Ethanol is metabolized to acetaldehyde, and is rapidly converted to acetate, it leads to the production of ROS, which deranges the cell membrane resulting in the leakage of specific marker enzymes into the circulation (31). In our studies, ethanol induced rats showed reduced body weight gain as compared to the control groups. The body weight of rats was enhanced on supplementation with azelaic acid to ethanol fed rats revealing the beneficial effects of azelaic acid against alcohol induced hepatotoxicity.

Serum aminotransferases (AST, ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), are extremely sensitive markers for the diagnosis of liver damage (32). Elevation in the activities of these cytosolic enzymes in the blood stream are indicator marker of hepatotocyte damage. In our present study ethanol fed rats showed a significant increase in the activities of AST, ALT, ALP, GGT in the serum indicating increased permeability, necrosis and hepatic damage, whereas azelaic acid supplementation decreased the activities of serum liver marker enzymes, showing the hepatoprotective effect of azelaic acid.

Alcohol dehydrogenase (ADH) and Aldehyde dehydrogenase (ALDH) are two catalytic enzymes involved in alcohol metabolism. Altered ratio of NAD/NADH inhibits gluconeogenesis and fatty acid oxidation thereby promoting fatty liver. CYP2E1, which is upregulated in chronic alcohol consumption, generates free radicals through the oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) to nicotinamide adenine dinucleotide phosphate (NADP) (33). Polymorphism of these alcohol metabolizing enzymes may contribute to modifications in the ethanol elimination rate. Chronic intake of alcohol increases

the ADH activity and decreases ALDH enzyme activity causing the accumulation of acetaldehyde in the blood. Acetaldehyde not only promotes glutathione (GSH) depletion and free radical-mediated toxicity (34) but also damages the hepatotocytes by forming acetaldehyde protein adducts, which in turn contributes to cirrhosis and HCC (35).

Oxygen derived free radicals are principal mediators of tissue injury and inflammation and are known to be cytotoxic and may therefore cause hepatocyte injury and necrosis (36). Ethanol is known to exert toxic effects on the liver and other extra-hepatic tissues. Lipid peroxidation is one of the most potent toxic effects during ethanol oxidation. Lipid peroxidation generates increased levels of byproducts such as TBARS, LOOH and CD which can damage the liver tissue (37). In our present study, ethanol treated rat showed increased levels of lipid peroxidative by products, whereas treatment with azelaic acid decreased the extent of lipid peroxidation in the liver tissue. Ethanol has the capability to disturb the balance between the pro and antioxidant systems of the organism. Increased generation of oxygen derived free radicals produced by ethanol leads to diminished endogenous antioxidants and a consequent induction of cytochrome P4502E1 (2E1) in Kupffer cells leading to further free radicals release from ethanol metabolism. Therefore, oxidative stress in alcoholic liver disease is most presumably mediated by both an increase in pro-oxidant production and a decrease in antioxidant defenses (38).However azelaic acid supplementation, due to its inherent antioxidant properties, markedly reduced the levels of ROS and lipid peroxidation byproducts.

A variety of enzymatic and non-enzymatic mechanisms have evolved to protect cells against ROS, including the superoxide dismutases, which remove ($O^{\cdot}_2$), catalase and the glutathione (GSH) peroxidase system which remove H_2O_2. Increased generation of oxygen derived free radicals produced by ethanol leads to diminished endogenous antioxidants. Enzymatic antioxidants such as SOD, CAT, GPx GR and GST are the first line of defense against oxidative injury. In addition, GSH the major non-enzymatic antioxidant plays a central role in coordinating the antioxidant defense processes, which is involved in the maintenance of normal cell structure and function, because of its involvement in redox and detoxification reactions. A decrease in SOD activity generally reflects the inability of a tissue to scavenge excess superoxide anions leading to oxidative stress. CAT is responsible for the catabolism of H_2O_2. Chronic ethanol consumption diminished the hepatic activities of SOD and CAT. Decrease in the activities of these antioxidants may lead to accumulation of $O_2\cdot-$ and H_2O_2, which in turn generates hydroxyl radicals, resulting in the initiation and propagation of lipid peroxidation (39), that is indicated by an increased in hepatic MDA levels. The decrease in GR in ethanol treated rats is indicative of impaired reduction of GSSG to GSH due to the depletion of the reducing equivalent NADPH, which is a co-substrate required for GR activity (40). Chronic ethanol treatment reduced the activity of GST thereby impairing the tissue detoxifying potential against ROS generation. Moreover, GPx and GST play a crucial role in scavenging ROS and/ or free radicals, and acute or chronic ethanol ingestion causes a decrease in the activities of GPx and GST in the tissue of rats.

In response to alcohol increased hepatic lipid peroxidation, decreased hepatic GSH concentrations is reported in various species such as man, rat, and baboon. This implies increased superoxide production because of singlet oxygen and hydroxyl radicals and these oxygens derived free radicals cause the peroxidation of unsaturated lipids in cellular membranes (41). Acetaldehyde may induce free radicals generation in the hepatocytes depleting mitochondrial reduced glutathione (GSH). Eariler report stated that chronic ethanol intake decreased the levels of GSH. This may be because GSH is utilized and oxidized for scavenging the ROS generated by ethanol metabolism (42). GSH is an antioxidant, preventing damage to important cellular components caused by ROS. However, supplementation with azeliac acid significantly elevated the antioxidant enzymes, Azelaic acid due to its inherent antioxidant properties, could have spared the antioxidant enzymes by getting oxidized themselves.

The function of vitamin E is to trap peroxyl radicals and break the chain reaction of lipid peroxidation (43).Vitamin C is a water soluble antioxidant which decreases lipid peroxidation either directly or indirectly by regenerating vitamin E, the major lipid-soluble antioxidant. (44) reported decreased hepatic α-tocopherol content after chronic ethanol ingestion in rats. Hepatic lipid peroxidation is significantly greater after long-term ethanol feeding in rats receiving a low vitamin E diet. However, supplementation with azelaic acid to ethanol treated rats resulted in significantly increased levels of non-enzymatic antioxidants which in turn reveals the strong antioxidant potential of azelaic acid in counteracting free radical mediated injury induced by ethanol. In this context, the antioxidant potential

of azelaic acid is believed to be mainly due to its redox properties, allowing it to act as a reducing agent. Azelaic acid supplementation increased the activities of the antioxidant enzymes and reduced lipid peroxidation in our study. In this context, protective effect of azelaic acid against high fat induced oxidative stress in liver, kidney, and heart of C57BL/6J mice was reported by (24).

Histopathological findings suggest that ethanol induces alterations in liver morphology and renal dysfunction. The ethanol treated rats showed changes in the structural integrity of the hepatocytes, massive centrilobular necrosis, central vein dilation, ballooning degeneration and inflammatory cellular infiltration of liver associated with liver damage (45) when the rats were cotreated with azelaic acid and ethanol, the liver showed normal architecture with mild congestion of the central vein revealing the potent effect of azelaic acid against beneficial ethanol-induced hepatoxicity.

CONCLUSION

These observations suggest that ethanol-induced liver damage can be reduced by supplementing azelaic acid as documented by the apparent improvement in the liver function. The hepatoprotective effect of azelaic acid is most likely mediated by preventing ethanol-induced oxidative stress. Among the three doses of azelaic acid used, 80mg/kg. b. w was found to be the most effective against ethanol induced toxicity. However, further detailed studies on the mechanism of action and needful targeting of specific signaling pathways are warranted in future.

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CONFLICTS OF INTEREST

There authors report no conflicts of interest.

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