Effect of L-ornithine L-aspartate on Liver Injury Due to Acute Ethyl Alcohol Intoxication in Rats

HM Durgun[1], A Ozhasenekler[1], R Dursun[1], MK Basarali[2], G Turkcu[3], M Orak[1], M Ustundag[1], C Guloglu[1]

ABSTRACT

Objective: *Ethyl alcohol is a substance that is widely used worldwide and known to exert toxic effects on liver. In this study, we aimed to examine the effect of L-ornithine L-aspartate (LOLA) on the toxicity of a single dose of ethyl alcohol in rats.*

Subjects and Method: *We used 32 randomly selected male Sprague-Dawley rats weighing 200–250 g. The rats were grouped into four groups with each group containing eight rats: Group 1: the control group, Group 2: the ethyl alcohol group, Group 3: the LOLA group and Group 4: the ethyl alcohol+LOLA group. Ethyl alcohol was administered orally through a nasogastric tube at a dose of 6 g/kg after diluting with distilled water. One hour after ethyl alcohol administration, LOLA was administered to pre-specified groups orally through a nasogastric tube at a dose of 200 mg/kg after diluting with distilled water. Liver tissue and blood samples were obtained from all rats 24 hours later to study total antioxidant capacity (TAC), total oxidant status (TOS) and oxidative stress index (OSI) levels in liver samples, and aspartate aminotransferase (AST), alanine transferase (ALT), TAC, TOS and OSI levels in blood samples.*

Results: *Serum TAC, TOS and OSI levels were higher in the groups that were administered ethyl alcohol. In addition, tissue TAC level was higher and TOS and OSI levels were lower in groups that were given ethyl alcohol. No significant changes were observed in serum and tissue TAC, TOS, OSI, ALT and AST levels in the LOLA administered groups.*

Conclusion: *This study showed that LOLA was not biochemically effective and exerts no oxidative stress reducing activity in liver injury due to acute ethyl alcohol toxicity.*

Keywords: Ethyl alcohol, L-ornithine L-aspartate, oxidative stress index, total oxidant status

Efecto de la L-ornitina L-aspartato en la Lesión Hepática Debido a la Intoxicación Aguda por Alcohol Etílico en Ratas

HM Durgun[1], A Ozhasenekler[1], R Dursun[1], MK Basarali[2], G Turkcu[3], M Orak[1], M Ustundag[1], C Guloglu[1]

RESUMEN

Objetivo: *El alcohol etílico es una sustancia ampliamente utilizada en todo el mundo y conocida por sus efectos tóxicos sobre el hígado. El objetivo de este estudio fue analizar el efecto de la L-ornitina L-aspartato (LOLA) en la toxicidad de una dosis única de etanol en ratas.*

Sujetos y método. *Utilizamos 32 ratas machos Sprague-Dawley de 200 – 250 g de peso seleccionadas al azar. Las ratas fueron divididas en cuatro grupos de ocho ratas cada uno. Grupo 1: grupo control; Grupo 2: grupo de alcohol etílico; Grupo 3: grupo de LOLA; y Grupo 4: grupo de alcohol etílico + LOLA. El alcohol etílico fue administrado por vía oral a través de una sonda nasogástrica a una dosis de 6 g/kg después de su dilución con agua destilada. Una hora después de la administración del alcohol etílico, se administró LOLA a grupos predefinidos por vía oral a través de una sonda nasogástrica a una dosis de 200 mg/kg después de su dilución con agua destilada. Se obtuvieron muestras de tejido del hígado y muestras de sangre de todas las ratas 24 horas más tarde, a fin de estudiar los niveles de la capacidad antioxidante total (CAT), el estado oxidante total (EOT) y los niveles de índice de estrés oxidativo (IEO) en las muestras de hígado, y los niveles de aspartato aminotransferasa (AST), alanino transferasa (ALT), CAT, EOT e IEO en las muestras de sangre.*

From: [1]Emergency Medicine Department, [2]Biochemistry Department and [3]Pathology Medicine Department, Medicine Faculty, University of Dicle, Diyarbakır, Turkey.

Correspondence: Dr HM Durgun, Emergency Medicine Department, Medicine School, University of Dicle 21280, Diyarbakır, Turkey. E-mail: hmdurgun@mynet.com

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Resultados: *Los niveles de suero CAT, EOT y IEO fueron más altos en los grupos a los que se les administró alcohol etílico. Además, el nivel de tejido en CAT fue más alto, y los niveles de EOT e IEO fueron más bajos en los grupos que recibieron alcohol etílico. No se observaron cambios significativos en los niveles séricos y tisulares de CAT, EOT, IEO, ALT, y AST en los grupos a los que se le administró LOLA.*
Conclusión: *Este estudio demostró que LOLA no fue bioquímicamente eficaz y no ejerce actividad de reducción del estrés oxidativo alguna en la lesión del hígado debida a la toxicidad aguda del alcohol etílico.*

Palabras claves: Alcohol etílico, L-ornitina L-aspartato, índice de estrés oxidativo, estado antioxidante total

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INTRODUCTION

Ethyl alcohol is a substance with known toxic effects on the liver, and has been abused for centuries worldwide (1). Ethyl alcohol abuse and its steadily increasing prevalence, particularly among young people, usually with consumption at the toxicity limits, have been considered as growing threats to public health in developed countries. It has been reported that in Australia, nearly 90% of youngsters 15–16 years of age have already tried alcohol twice at the time of the study, 29% of these ingestions being at the intoxication doses. More than 32% of young people have suffered acute alcohol intoxication and related problems in the United States of America [USA] (2).

Acute ethyl alcohol intoxication is especially a serious problem for emergency departments. One domestic study reported that 6.6% of intoxication cases that presented to the emergency department between 2002 and 2003 were alcohol intoxication (3). One other study reported that 7% of motor cycle crashes were caused by drivers under the effect of alcohol (4). Alcohol abuse also has economic consequences. In Italy, for example, the economic burden of alcohol-related diseases makes up 5–6% of gross national product (GNP), while the corresponding figure is 2–5% for the rest of Europe (2).

Alcoholic liver disease may be asymptomatic or manifest itself as reversible fatty liver, acute alcoholic hepatitis, or cirrhosis (5). Acute ethyl alcohol intoxication is usually characterized by alanine transferase (ALT) and aspartate aminotransferase (AST) elevation. Acute and chronic ethyl alcohol ingestion increase production of reactive oxygen radicals, reduce cellular antioxidant levels, and heighten oxidative stress in liver and many other tissues (6–10). Oxidative stress is related to free oxygen radicals that injure cells and tissues and can be defined as an increase of oxidative capacity and/or a decrease of antioxidative capacity (11). Total antioxidant capacity (TAC), total oxidant status (TOS) and oxidative stress index (OSI) levels are typically used to determine oxidative stress (12).

L-ornithine L-aspartate (LOLA) is a safe and easily tolerated substance that acts by detoxification of ammonia in liver cirrhosis, chronic hepatic encephalopathy and subclinical encephalopathy (13). The known effect of LOLA is to induce hepatic urea synthesis, increase glutamine production in muscles and regulate the relationship between branched aromatic amino acids. For this purpose, it is believed to be beneficial in hepatic encephalopathy and it is commonly used in this disorder (14, 15). L-ornithine L-aspartate also exerts a hepatoprotective effect by stabilization of peroxidant/antioxidant balance of liver cells v*ia* an antioxidative mechanism (16).

The experimental animal model in which acute liver toxicity is created by acute ethyl alcohol intoxication is characterized by biochemical and histopathological definitions and the ensuing liver injury shows similarities with human liver injury from both biological and morphological aspects (17–19). Although there are many studies suggesting the effectiveness of LOLA in hepatic encephalopathy, data indicating its benefit in acute liver toxicity are insufficient (20). We herein aimed to assess the effect of LOLA on toxicity of a single dose of ethyl alcohol in rats.

SUBJECTS AND METHOD

Animals and experimental protocol

This study used 32 randomly selected male Sprague-Dawley rats weighing 200–250 g that were cared at Dicle University Health Sciences and Research Center (Diyarbakır, Turkey). The rats were housed in wooden cages with a size of 14 × 9 × 8 cm. Before the experiment, all rats were fed with standard rat chow and water *ad libitum* and were kept in an air-conditioned room at 21 °C with a 12-hour light:dark cycle. All animals were treated humanely. Animals were subjected to an adequate fasting period before the experiment. Dicle University Local Ethics Committee for Animal Experiments (Diyarbakır, Turkey) approved the study.

The rats were grouped into four groups, each containing eight rats: Group 1: control group, Group 2: ethyl alcohol group, Group 3: LOLA group and Group 4: ethyl alcohol+LOLA group.

Ethyl alcohol was administered orally *via* a nasogastric tube after diluting with distilled water at a single dose of 6 g/kg which is the maximum toxic dose at which no or minimal death occurs (17, 18). One hour after ethyl alcohol administration, LOLA (21) was administered to pre-specified groups orally through a nasogastric tube at a dose of 200 mg/kg after diluting with distilled water. All rats were anaesthetized with ketamine hydrochloride (50 mg/kg, intramuscularly) 24 hours after the treatment. All rats were then laid down in the supine position for the surgical procedure. Laparotomy *via* midline

incision was performed in all rats. Next, liver tissue and blood samples were taken from each animal. At the end of the procedure, all rats were killed with the exsanguination method.

Biochemical analysis and oxidant/antioxidant parameters
Sera obtained after centrifuge of the blood samples taken *via* intracardiac route at maximum amount were preserved at -70 °C until the time of study for biochemical parameters. Liver tissue was homogenized for biochemical examinations and the supernatant portion of the homogenate that was obtained after the centrifuge procedure was properly transferred into Eppendorf tubes with a plastic cap. These transferred homogenates were kept at -70 °C until the time of study. Serum AST and ALT were determined by the Abbott Architect c16000 Autoanalyzer and expressed as U/L.

Biochemical steps and analyses
Blood samples were centrifuged at 3000 rpm for 10 minutes to obtain plasma samples that were used for TAC. Liver tissue samples were prepared at 4 °C for estimating tissue levels of oxidant and antioxidant substances. Tissue samples were weighed and cut into small pieces. They were then homogenized in 10 volumes of ice-cold phosphate buffer solution (PBS; 50 mM/L, pH 7.0) using a homogenizer (Ultra-Turrax T8 dispersing homogenizator; Staufen, Germany). The obtained homogenate was centrifuged at 15 000 rpm for 10 minutes at 4 °C to obtain supernatant samples to use for determination of TOS and TAC.

Measurement of the TAC: TAC of supernatant fractions was determined using a novel automated measurement method developed by Erel (22). In this method, hydroxyl radical, which is the most potent biological radical, is produced. In the assay, ferrous ion solution, which is present in Reagent 1, is mixed with hydrogen peroxide, which is present in Reagent 2. The sequentially produced radicals produced by the hydroxyl radical, such as the brown coloured dianisidinyl radical cation, are also potent radicals. Using this method, antioxidative effect of the sample on potent free radical reactions induced by the produced hydroxyl radical is measured. The assay has excellent precision values that are below 3%. The results are expressed as nmol Trolox Equiv./mg protein.

Measurement of TOS: TOS of supernatant fractions was determined using a novel automated measurement method developed by Erel (23). Oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules that are abundant in the reaction medium. The ferric ion makes a coloured complex with xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of nmol H_2O_2 Equiv./mg protein.

Determination of oxidative stress index
Per cent ratio of TOS level to TAC level was defined as OSI. Oxidative stress index value was calculated with the following formula (24): OSI (Arbitrary Unit) = TOS (nmol H_2O_2 Equiv./mg protein)/TAC (nmol Trolox Equiv./mg protein). The results are expressed as Arbitrary Unit.

Statistical analysis
Study data were analysed with SPSS 11.0. All groups showed normal distribution and thus nonparametric tests were used to analyse the study data. A one-way analysis of variance test was performed, and post hoc multiple comparisons were done with the Bonferroni method. The Chi-squared (Fisher's exact) test was used for analysis of categorical data. The Spearman's rho test was used for the correlation analysis. Results are presented as mean ± SD. A $p < 0.05$ was considered to be statistically significant.

RESULTS

The groups significantly differed with respect to serum ALT, AST levels and liver tissue and serum TAC, TOS, and OSI levels. Serum AST, ALT, liver tissue and serum TOS, TAC and OSI levels are shown on Table 1.

Inter-group comparisons with respect to serum ALT and AST levels are shown in Table 2. Inter-group comparison of serum ALT level revealed that it was significantly higher in Group 2 and Group 4 compared to Group 1 and Group 3 ($p < 0.001$). Serum AST level was significantly higher in Group

Table 1: Results of the biochemical studies

	Parameters	Group 1	Group 2	Group 3	Group 4	*p*
Serum	**AST (IU/L)**	117.00 ± 16.13	188.75 ± 58.42	144.25 ± 24.50	162.75 ± 31.52	**0.004**
	ALT (IU/L)	49.00 ± 8.00	87.12 ± 7.47	54.25 ± 7.66	78.62 ± 15.64	**< 0.001**
Liver	**TOS (µmol/L)**	113.21 ± 13.19	65.57 ± 28.36	134.34 ± 27.75	70.92 ± 12.49	**< 0.001**
	TAC (mmol/L)	3.63 ± 0.46	5.90 ± 1.56	3.22 ± 0.65	4.73 ± 1.46	**< 0.001**
	OSI (%)	3.14 ± 0.45	1.12 ± 0.39	4.33 ± 1.25	1.55 ± 0.37	**< 0.001**
	TOS (µmol/L)	23.70 ± 7.04	126.03 ± 27.58	35.16 ± 13.58	144.36 ± 34.04	**< 0.001**
Serum	**TAC (mmol/L)**	0.49 ± 0.10	0.84 ± 0.26	0.43 ± 0.15	0.71 ± 0.33	**< 0.001**
	OSI (%)	5.01 ± 1.67	16.05 ± 5.41	8.38 ± 3.55	13.90 ± 3.74	**< 0.001**

AST: aspartate aminotransferase; ALT: alanine aminotransferase; TOS: total oxidant status; TAC: total antioxidant capacity; OSI: oxidative stress index

Table 2: Inter-group comparisons with respect to serum ALT and AST levels

Parameter	Groups		*p*-value
ALT	**I** (49.00 ± 8.00)	**II** (87.12 ± 7.47)	< 0.001
		III (54.25 ± 7.66)	1.000
		IV (78.62 ± 15.64)	< 0.001
	II (87.12 ± 7.47)	**III** (54.25 ± 7.66)	< 0.001
		IV(78.62 ± 15.64)	0.658
	III (54.25 ± 7.66)	**IV** (78.62 ± 15.64)	< 0.001
AST	**I** (117.00 ± 16.13)	**II** (188.75 ± 58.42)	0.003
		III (144.25 ± 24.50)	0.866
		IV (162.75 ± 31.52)	0.106
	II (188.75 ± 58.42)	**III** (144.25 ± 24.50)	0.124
		IV(162.75 ± 31.52)	0.978
	III (144.25 ± 24.50)	**IV** (162.75 ± 31.52)	1.000

AST: aspartate aminotransferase; ALT: alanine aminotransferase

2 compared to Group 1 ($p < 0.001$). There were no significant differences between the other groups. Inter-group comparisons with respect to TAC, TOS and OSI levels are shown in Table 3. Serum TAC level was significantly higher in Group 4 than Group 1 *(*$p < 0.001$) and in Group 2 and 4 than Group 3 ($p = 0.004$ and $p < 0.001$, respectively). Serum TOS level was significantly higher in Group 2 and Group 4 compared to both Group 1 and Group 3 ($p < 0.001$). Serum OSI level was significantly higher in Group 2 and Group 4 compared to Group 1 ($p < 0.001$), while it was significantly higher in Group 2 than Group 3 ($p = 0.002$).

Table 3: Inter-group comparisons with respect to TAC, TOS and OSI levels

Parameter	Groups		*p*-value
Serum TAC	**I** (0.49 ± 0.10)	**II** (0.84 ± 0.26)	0.012
		III (0.43 ± 0.15)	1.000
		IV (0.71 ± 0.33)	< 0.001
	II (0.84 ± 0.26)	**III** (0.43 ± 0.15)	0.004
		IV (0.71 ± 0.33)	0.205
	III (0.43 ± 0.15)	**IV** (0.71 ± 0.33)	< 0.001
Serum TOS	**I** (23.70 ± 7.04)	**II** (126.03 ± 27.58)	< 0.001
		III (35.16 ± 13.58)	1.000
		IV (144.36 ± 34.04)	< 0.001
	II (126.03 ± 27.58)	**III** (35.16 ± 13.58)	< 0.001
		IV (144.36 ± 34.04)	0.753
	III (35.16 ± 13.58)	**IV** (144.36 ± 34.04)	< 0.001
Serum OSI	**I** (5.01 ± 1.67)	**II** (16.05 ± 5.41)	< 0.001
		III (8.38 ± 3.55)	0.536
		IV (13.90 ± 3.74)	< 0.001
	II (16.05 ± 5.41)	**III** (8.38 ± 3.55)	0.002
		IV (13.90 ± 3.74)	1.000
	III (8.38 ± 3.55)	**IV** (13.90 ± 3.74)	0.045

TAC: total antioxidant capacity; TOS: total oxidant status; OSI: oxidative stress index

Inter-group comparisons with respect to tissue TAC, TOS and OSI levels are shown in Table 4. Tissue TAC level was significantly higher in Group 2 than Group 1 and Group 3 ($p = 0.001$ and $p < 0.001$, respectively), while it was significantly lower in Group 3 compared to Group 4 ($p = 0.046$). Tissue TOS level was significantly lower in Group 2 and 4 than Group 1 ($p = 0.001$ and $p = 0.003$, respectively), Group 3 than Group 2 ($p < 0.001$), and Group 4 than Group 3 ($p < 0.001$). Tissue OSI level was significantly lower in Group 2 and Group 4 than Group 1 ($p < 0.001$ and $p = 0.001$, respectively), Group 2 than Group 3 ($p < 0.001$), and Group 4 than Group 3 ($p < 0.001$).

Table 4: Inter-group comparisons with respect to tissue TAC, TOS and OSI levels

Parameter	Groups		*p*-value
Tissue TAC	**I** (3.63 ± 0.46)	**II** (5.90 ± 1.56)	0.001
		III (3.22 ± 0.65)	1.000
		IV (4.73 ± 1.46)	0.281
	II (5.90 ± 1.56)	**III** (3.22 ± 0.65)	< 0.001
		IV(4.73 ± 1.46)	0.218
	III (3.22 ± 0.65)	**IV** (4.73 ± 1.46)	0.046
Tissue TOS	**I** (113.21 ± 13.19)	**II** (65.57 ± 28.36)	0.001
		III (134.34 ± 27.75)	0.377
		IV (70.92 ± 12.49)	0.003
	II (65.57 ± 28.36)	**III** (134.34 ± 27.75)	< 0.001
		IV(70.92 ± 12.49)	0.001
	III (134.34 ± 27.75)	**IV** (70.92 ± 12.49)	< 0.001
Tissue OSI	**I** (3.14 ± 0.45)	**II** (1.12 ± 0.39)	< 0.001
		III (4.33 ± 1.25)	0.015
		IV (1.55 ± 0.37)	0.001
	II (1.12 ± 0.39)	**III** (4.33 ± 1.25)	< 0.001
		IV(1.55 ± 0.37)	1.000
	III (4.33 ± 1.25)	**IV** (1.55 ± 0.37)	< 0.001

TAC: total antioxidant capacity; TOS: total oxidant status; OSI: oxidative stress index

DISCUSSION

Ethyl alcohol (CH_3CH_2OH) is a water soluble substance. It is rapidly absorbed following oral administration and reaches peak concentration 30–60 minutes after administration (25). Ten per cent of the ingested amount is metabolized by gastric alcohol dehydrogenase while still in the stomach and the remaining 90% is metabolized into acetaldehyde by three enzymatic pathways in the liver (26). Since 90% of its metabolism takes place in the liver, its chronic use may progress into hepatosteatosis, liver failure and cirrhosis, while acute consumption in high doses may lead to acute alcoholic hepatitis. Therefore, acute ethyl alcohol intoxication and the related acute alcoholic hepatitis pose serious problems to emergency departments.

Injured hepatocytes release AST and ALT into the bloodstream. Thus, the classical laboratory findings of hepatotoxicity are elevation in AST and ALT (27). Many studies experimentally inducing liver injury with an ethanol dose of 5 mg/kg have reported an increase especially in ALT level (17–19). Also, in our study, serum ALT and AST levels were markedly higher in groups administered ethyl alcohol.

Reactive oxygen radicals play an important role in ethyl alcohol-induced hepatotoxicity (6). Hepatic oxidative stress has been found to increase in many studies that experimentally induced liver injury in mice and rats with administration of

ethyl alcohol at a dose of 5–6 mg/kg (17, 18). Serum TOS levels have been reported higher in non-alcoholic hepatosteatosis (28). In a study that induced oxidative stress in liver tissue by administering humic acid in rats, serum TAC, TOS and OSI levels were found higher in the humic acid group compared to the control group (29). Ozhasenekler *et al* reported an increase in TOS level, a decrease in TAC level and a marked increase in OSI level in acute paracetamol-induced liver injury in rats (30). In alcohol administered groups in our study, serum TAC, TOS, OSI and tissue TAC levels were higher but tissue TOS and OSI levels were lower.

L-ornithine L-aspartate reduced ethyl alcohol-induced oxidative stress in a study where oxidative stress was caused in testes by administering ethyl alcohol (31). Another study induced hepatic injury by administering thioacetamide in rats and examined the protective activity of LOLA. It revealed that LOLA significantly reduced hepatic injury and decreased oxidative stress causing liver injury (32). Ozhasenekler *et al* reported that LOLA was biochemically ineffective for protecting against acetaminophen-induced liver injury (30). In our study, LOLA did not have any reducing effect on oxidative injury.

In conclusion, our study demonstrated that LOLA was biochemically ineffective for protecting against liver injury induced by acute ethyl alcohol intoxication.

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