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Full Length Research Paper

Cactus (*Opuntia ficus indica f. inermis*) fruit juice protects against ethanol-induced hematological and biochemical damages in rats

Hichem Alimi[1,2]*, Najla Hfaeidh[3], Zouhour Bouoni[1], Sakhria Mbarki[3], Mohsen Sakly[2] and Khémais Ben Rhouma[2]

[1]Unité de Biochimie Macromoléculaire et Génétique, Faculté des Sciences, 2112 Gafsa, Tunisia.
[2]Laboratoire de Physiologie Intégrée, Faculté des Sciences, 7021 Jarzouna, Bizerte, Tunisia.
[3]Laboratoire d'Ecophysiologie Animale, Faculté des Sciences, 3018 Sfax, Tunisia.

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A putative beneficial effect of *Opuntia ficus indica f. inermis* prickly pear juice (OFIj) was tested on ethanol-induced hematological and biochemical damages in rats. Our results show that chronic ethanol treatment (300 mg/100 g body weight for 90 days) of Wistar rats (group 2) significantly reduced red blood cells (RBC) and platelet (Plt) counts, hemoglobin (Hb) content, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) whereas white blood cells (WBC) counts and the mean corpuscular volume (MCV) significantly increased as compared to the controls rats treated with same distilled water (group 1). In addition, serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and γ-glutamyl transpeptidase (γ-GT) as well as urea, creatinine, cholesterol and triglycerides concentrations significantly increased in ethanol-fed rats. Furthermore, serum, hepatic and renal lipid peroxidation levels were also increased in animals given ethanol compared to the controls. In alcoholic rats co-treated with 4 ml OFIj / 100 g b.w. (group 3), all the above cited parameters were maintained to near-normal values. In group 4 only 4 ml OFIj / 100 g b.w. was given, no changed parameters was shown. Therefore, OFIj appeared to be a promising agent for protection against ethanol toxicity.

Key words: *Opuntia*, alcohol, blood, liver, kidney, toxicity.

INTRODUCTION

Epidemiological, experimental and clinical investigations have shown a strong consistent relationship between alcohol abuse and liver diseases, hypertension, blood anomalies and other disorders (Husain et al., 2001; Russo et al., 2004). The ethanol-related diseases are instigated by excess production of acetaldehyde, hydrogen peroxide (H_2O_2) and reactive oxygen species (ROS), resulting from ethanol metabolism via the alcohol dehydrogenase (ADH) and the cytochrome P450-2E1 (CYP2E1) enzymes (Nordmann et al., 1992; Cederbaum et al., 2009). In clinical investigations, hematological and biochemical parameters are used to follow the evolution of the diseases resulting from ethanol abuse. Numerous studies demonstrate that fruits of some berry plants biosynthesize phytochemicals possessing antioxidant activity which could be used as a natural source of free radical scavengers (Yurt and Celik, 2011; Erukainure et al., 2011). In this respect, we were interested in the protective potential of cactus plants.

Native to Mexico, cactus plant is widespread through

*Corresponding author. E-mail: alimihichem@yahoo.fr. Tel: (00216) 96 71 3333. Fax: (00216) 76211026.

Abbreviations: OFIj, *Opuntia ficus indica f. inermis* prickly pear juice; **RBC,** red blood cells; **Plt,** platelet; **Hb**, hemoglobin; **MCH,** mean corpuscular hemoglobin; **MCHC,** mean corpuscular hemoglobin concentration; **WBC,** white blood cells; **MCV,** mean corpuscular volume; **ALT,** alanine aminotransferase; **AST,** aspartate aminotransferase; **ALP,** alkaline phosphatase; **LDH,** lactate dehydrogenase; **γ-GT,** γ-glutamyl transpeptidase.

Table 1. Scheme of drugs treatments.

Group	Treatment	
	At 9 h:00 in the morning	At 13 h:00 after noon
Control	Water (10 ml/kg b.w)	Water (10 ml/kg b.w)
EtOH	Water (10 ml/kg b.w)	Ethanol (10 ml/kg b.w)
OFIj+EtOH	OFIj (4 ml/100 g b.w	Ethanol (10 ml/kg b.w)
OFIj	OFIj (4 ml/100 g b.w)	Water (10 ml/kg b.w)

Water: distilled water, ethanol (10 ml/kg b.w.) = Ethanol (3 g/kg b.w).

out South America, Australia, South Africa, and the whole Mediterranean area (Galati et al., 2003; Tesoriere et al., 2004). *Opuntia ficus indica f. inermis* species grows throughout Tunisia and is mainly cultivated for its sweet and juicy fruit (prickly pear), which was shown to be rich in antioxidant compounds such as polyphenols, flavonoids, betalains, and ascorbic acid. *Opuntia* fruits were found to display interesting properties such as antiulcerrogenic (Galati et al., 2003), antioxidant (Kuti, 2004), and neuroprotective (Dok-Go et al., 2003). Moreover, prickly pear is used for the treatment of gastritis, hyperglycemia, arteriosclerosis, diabetes, and prostate hypertrophy (Agozzino et al., 2005). In Chinese medicine, cactus pear is used for inflammation and pain treatment (Zou et al., 2005). To our knowledge, there is no information hitherto about the effect of *O. ficus indica f. inermis* prickly pears or their juice on alcohol toxicity.

Therefore, the present study was undertaken to investigate the effects of *O. ficus indica f. inermis* prickly pear juice on hematological and biochemical disorders induced by ethanol treatment in rats.

MATERIALS AND METHODS

Chemicals

Ethanol 96.2% was purchased from Carlo Erba Reagents. Trichloroacetic acid (TCA), butylated hydroxytoluene (BHT), 2-thiobarbituric acid (TBA) and all other chemical products used in this study were purchased from Sigma Chemicals (Aldrich Chemical Company).

Preparation of *O. ficus indica f. inermis* fruit juice (OFIj)

Mature prickly pears of *O. ficus indica f. inermis* species (purple-skinned) were collected from local area. The whole unpeeled fruit (30 kg) was washed, ground by a Musermax double bladed mill and filtered through a colander (0.5 mm mesh size) to discard seeds. The resulting juice was centrifuged at 3000 × g for 10 min to remove hard fibers. The clarified juice (16.6 L) was then collected and stored at −21°C until use.

Animals

Adult male albino Wistar rats (n = 32) weighing 160 to 180 g were obtained from Pasteur Institute of Tunisia. Animals were quarantined and allowed to acclimatize for a week prior to experimentation. The animals were handled under standard laboratory conditions of temperature (22 ±2°C), relative humidity (70 ± 4%), and a 12 h light/dark cycle. Animals were fed with commercial pellets and given tap water *ad libitum*. Experiments were carried out according to the Tunisian code of practice for the care and use of animals for scientific purposes.

Experimental design

After acclimation period, rats were randomly divided into four groups of eight animals each, initially weighted then treated for 90 days with two consecutive intra-gastric intubations per day of (1) pure water (control group), (2) ethanol (prepared at 30% in distilled water), (3) both OFIj and ethanol or (4) only OFIj extract as described in Table 1. Food intake was daily monitored in each group during the entire treatment period. At the end of the experimental period, the final body weight of the animals, absolute liver and kidney weights were determined.

Hematological parameters

At the end of the experimental period, all animals were sacrificed by cervical dislocation. Blood samples were immediately collected in two tubes; the first was dry and the second was heparinized. The last tubes served to determine hematological parameters (red blood cell number (RBC), hemoglobin concentration (Hb), hematocrit value (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet (Plt) and white blood cell number (WBC)) using a hematology analyzer Coulter MAXM (Beckman Coulter, Inc., Fullerton, USA).

Evaluation of biochemical parameters

Serum samples were obtained by the centrifugation of the blood collected in the dry tubes at 1000 x *g* for 10 min at 4°C, and were then stored at -20°C until analyses. The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transferase (γ-GT), cholesterol, triglyceride, total protein, urea and creatinin were measured using provided BioMaghreb commercial kits.

Lipid peroxidation estimation

The liver and the kidney were quickly excised, rinsed with ice cold saline solution, weighted and homogenized (1:2, w/v) in 50 mmol/L phosphate buffer (pH 7.4) using an Ultra Turrax homogenizer and centrifuged at 4°C. The supernatant were frozen at -20°C in aliquots until analysis. Lipid peroxidation in the serum and tissue

Table 2. Effects of ethanol, OFIj or their combination (OFIj+EtOH) on weight gain, food intake and absolute liver and kidney weights.

Parameter	Experimental group			
	Control	EtOH	OFIj+EtOH	OFIj
Gain weights (%)	38.2±1.08	22.5±2.31**##	35.61±1.41	36.4±1.06
Food intake (g/rat/day)	13.6±0.97	9.2±0.31**#	11.32±0.68	11.9±0.47
A. liver weight (g)	8.67±0.25	10.86±0.14*#	8.37±0.32	8.48±0.41
A. kidney weight (g)	0.99±0.09	0.65±0.06*#	0.87±0.04	0.96±0.03

Values are expressed as means ± SD, for eight rats in each group. Significant differences were calculated at *p < 0.05, **p < 0.01 vs, control group and at #p <0.05, ##p < 0.01 vs, OFIj + ethanol group. A: Absolute.

Table 3. Hematological parameters of control and rats treated with ethanol, OFIj or their combination (EtOH + OFIj).

Parameter	Experimental group			
	Control	EtOH	OFIj+EtOH	OFIj
RBC (106/µl)	7.28±0.42	6.73±0.25*#	7.16±0.36	7.46±0.32
Hb (g/dl)	14.25±038	10.31±0.54*#	13.83±0.49	14.38±051
Ht (%)	44.81±1.23	37.65±1.41**##	42.37±1.32	43.92±1.21
MCV (mm^3/RBC)	57.43±2.34	65.72±3.41*#	54.68±2.21	56.83±2.89
MCH (pg/RBC)	20.16±0.67	17.83±0.38*#	19.81±0.49	20.34±0.58
MCHC (g/dl)	33.73±1.53	28.26±1.28**#	32.65±1.42	33.84±1.61
Plt (103/µl)	734.34±41.1	283.91±25.7**#	723.49±36.4	738.21± 49.3
WBC (103/µl)	10.67±0.23	15.43±0.91**#	11.23±0.43	10.53±0.16

Values are expressed as means ± SD, for eight rats in each group. Significant differences were calculated at *p < 0.05, **p < 0.01 vs, control group; and at #p <0.05, ##p <0.01vs, OFIj + ethanol group. RBC: Red blood cells, Hb: Hemoglobin, Ht: Haematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, Plt: Platelet and WBC: white blood cells.

homogenate was estimated by measuring thiobarbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content which is the end product of lipid peroxidation, according to the method of Ohkawa et al. (1979). In brief, 125 µl of samples were homogenized by sonication with 50 µl of TBS, 125 µl of TCA-BHT in order to precipitate proteins and centrifuged (1000 × g, for 10 min at 4°C). The obtained supernatant (200 µl) were mixed with 40 µl of HCl (0.6 M) and 160 µl of TBA dissolved in Tris and the mixture was heated at 80°C for 10 min. The absorbance of the resultant supernatant was read at 530 nm. The amount of MDA was calculated using an extinction coefficient of $156 \times 10^5\ mM^{-1}\ cm^{-1}$.

Statistical analysis

All data were expressed as mean ± SD. Differences among the experimental groups were assessed by one-way ANOVA followed by Duncan's test. Values were considered statistically significant when $p < 0.05$.

RESULTS

Effect of OFIj and/or ethanol on food intake, body and organ weights

Food intake, body growth and kidney weight were significantly lower in ethanol-treated rats, as compared to the controls (Table 2). By contrast, chronic ethanol administration significantly increased ($p < 0.05$) liver weight as compared to the controls. When OFIj was administered together with ethanol, the adverse effects of ethanol upon these parameters were alleviated. Administration of OFIj alone did not cause any significant alteration of the studied parameters.

Hematological parameters

Values of hematological parameters are reported in Table 3. In rats receiving ethanol, erythrocytes number (RBC), hemoglobin concentration (Hb), hematocrit value (Ht), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets (Plt) number where significantly reduced as compared to the controls. In contrast, mean corpuscular volume (MCV) level and leucocytes number (WBC) were higher than that in the controls. No significant changes were observed in hematological parameters when animals were given both OFIj and ethanol, demonstrating a protective effect of the cactus fruit juice. Administration of OFIj alone to healthy rats did not cause any significant

Table 4. Enzyme activities in the serum of control and rats treated with ethanol, OFIj or their combination (EtOH + OFIj).

Enzyme (U/L)	Experimental group			
	Control	EtOH	OFIj+EtOH	OFIj
AST	134.8±2.1	214.7±5.8***##	153.6±4.1*	129.3±1.7
ALT	51.3±2.8	87.3±6.5**#	58.9±4.6*	50.8±4.8
ALP	58.5±4.2	94.6±5.7**#	64.1±4.7	59.6±5.1
LDH	928.4±21.3	1488.6±57.8***#	1106.2±41.7	935.7±52.9
γGT	2.34±0.12	5.32±0.36**#	3.14±0.28	2.31±0.16

Values are expressed as means ± SD, for eight rats in each group. AST: Aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; LDH: lacatate dehydrogenase and γGT: gamma glutamyl transferase. Significant differences were calculated at $^{*}p < 0.05$, $^{**}p < 0.01$, $^{*}p < 0.001$vs, control group; and at $^{\#}p < 0.05$, $^{\#\#}p < 0.01$vs, OFIj + ethanol group.

Table 5. Changes of serum biochemical parameters of the control and rats treated with ethanol, OFIj or their combination (EtOH + OFIj).

Parameter	Experimental group			
	Control	EtOH	OFIj+EtOH	OFIj
Cholesterol (mg/dl)	76.28±5.31	116.28±4.25**#	81.62±6.76	73.4±4.08
Triglyceride (mg/dl)	84.72±6.24	106.11±5.31**#	92.41±4.13	85.65±6.73
Total protein (g/dl)	6.98±0.48	5.15±0.41*#	6.37±0.32	6.95±0.81
Urea (mg/dl)	32.21±1.51	59.81±2.46*#	37.62±2.31	31.87±2.46
Creatinin (mg/dl)	0.42±0.07	0.86±0.05*#	0.51±0.09	0.38±0.04

Values are expressed as means ± SD, for eight rats in each group. Significant differences were calculated at *p < 0.05, **p < 0.01 vs, control group and at $^{\#}p < 0.05$ vs, OFIj + ethanol group.

alteration of the hematological parameters either.

Biochemical parameters

The biochemical parameters of the control and experimental groups are shown in Tables 4 and 5. It appears that chronic ethanol administration significantly increases activities of transaminases (AST, ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and gamma-glutamyl transferase (γGT) as compared to the control group (Table 4). Data also shows that chronic ethanol administration significantly increased serum concentrations of cholesterol, triglycerides, urea and creatinine, while proteins concentration decreased (Table 5). When OFIj was administered together with ethanol, values of all theses parameters were kept close to the control values. OFIj, when given alone, did not change these values either.

Lipids peroxidation

MDA levels in serum, hepatic and renal tissues were significantly higher ($p<0.01$) in ethanol-treated rats than in the controls (Table 6). When rats were given OFIj together with ethanol, MDA levels in serum, liver and kidney significantly reduced as compared to the ethanol group. No significant difference in MDA levels was observed when comparing rats receiving OFIj alone and controls.

DISCUSSION

The various biochemical and hematological parameters investigated in this study are useful indices for evaluating the putative protective effect of the cactus prickly pear juice on disturbances induced by ethanol in rats hematological and biochemical parameters.

The present study showed a reduction of body weight, food intake and kidney weight in ethanol-treated rats. By contrast, ethanol intake significantly increased liver weight. Our results are in agreement with those reported by Dinu et al. (2005) and Kasdallah-Grissa et al. (2007). Obvious decrease of body and kidney weights may not result from a mere decrease in food intake, but rather could be due to the toxicity of ethanol on the gastro-intestinal tract leading to a relative impairment of nutrients digestion or to inhibition of protein synthesis (Saravanan et al., 2006). The observed increase of liver weight in ethanol-fed rats was in agreement with the observations of Navder et al. (1997) who attribute this increase to lipids accumulation. In our study, OFIj significantly mitigates the effects of ethanol on food intake and on body, liver and kidney weights. OFIj

Table 6. Hepatic, renal and serum malondialdehyde (MDA) contents of control and rats treated with ethanol, OFIj or their combination (EtOH + OFIj).

Parameter	Experimental group			
	Control	EtOH	OFIj+EtOH	OFIj
Liver MDA	0.78±0.04	1.36±0.08***##	0.81±0.02	0.76±0.01
Kidney MDA	0.76±0.02	1.09±0.05**##	0.79±0.06	0.74±0.03
Serum MDA	0.82±0.05	1.47±0.04***##	0.85±0.04	0.79±0.02

Values are expressed as means ± SD, for eight rats in each group. MDA was expressed as nmol/mg protein. Significant differences were calculated at **p < 0.01, ***p < 0.001vs. Control group; and at #p <0.05, ##p <0.01vs. OFIj + ethanol group.

provides a wide range of natural antioxidants to ethanol-fed rats including polyphenols, flavonoids, ascorbic acid, carotenoids, and betalains compounds (Alimi et al., 2012a). This kind of antioxidants could protect body from ethanol-inducing oxidative damage.

It has been reported that ethanol causes hematological disturbances in various clinical and experimental studies (Kanbak et al., 2007; Padmini and Sundari, 2008). The present study demonstrates that chronic ethanol administration induced a decrease in the levels of rats Hb, Ht, MCH, MCHC and RBC and Plt numbers. The reduction in RBCs, Hb and Ht might be due to an inhibition of erythropoiesis and hemoglobin synthesis and to an increase in the rate of erythrocytes destruction (Maruyama et al., 2001). The observed decrease of RBCs, Hb, Ht values and the increase of MCV value in rats exposed to ethanol could be the manifestation of swollen erythrocytes and macrocytic anemia (Harold and Ballard, 1997). In agreement with the above observations our previous study (Alimi et al., 2012a) showed that chronic ethanol ingestion leads to a marked anemia in rats, evidenced by a large production of deformed erythrocytes, associated to an increase in erythrocyte hemolytic percentage. This last finding could explain the reduction of Hb, MCH and MCHC values observed in ethanol-fed rats. In this study the ethanol-treated rats also exhibited significantly higher WBC number than the control animals. The increase in WBC might be the activation marker of defense and immune system and showed that there were inflammations in the tissues (Maturu et al., 2011). The decrease in Plt count in ethanol-fed rats suggested a possible effect on blood coagulation and haemostasis blood system damage. In the present study, pretreatment of alcoholic rats with OFIj significantly mitigate the obvious hematological disturbances. In our previous study, we showed that OFIj was rich in polyphenols, flavonoids, ascorbic acid, carotenoids, and betalains compounds. Our previous study demonstrated that OFIj supplement, increased plasma scavenging activity of control and ethanol-fed rats and prevented the impairment of erythrocyte osmotic stability and morphologic aspect (Alimi et al., 2012a). Such abundance in antioxidant compounds might confer to OFIj an antioxidant activity and prevent blood cells from ethanol metabolites.

The results of the present study also showed that chronic ethyl alcohol ingestion caused a significant increase in serum levels of liver biochemical markers (AST, ALT, ALP, LDH and γGT). It has been shown that the disturbances in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells to the blood stream (Fan et al., 2009). The serum increase of these enzymes activities in ethanol-fed rats may be due to the increase of hepatocytes permeability damaged by ethanol metabolites, this obvious results was in agreement with the findings reported by Yurt and Celik (2011). More so, ethanol-inducing hepatocytes damage was confirmed in this study by increases of cholesterol, triglyceride and total protein contents in serum. Similar changes have been reported by Pari and Suresh (2008).

In addition, kidney is vulnerable to damage because of larger perfusion and the increased concentration of excreted compounds that occur in renal tubular cells (Dinu et al., 2005). Serum levels of creatinine and urea were used as indicators of renal function. Elevated blood urea is known to be linked with an increased protein catabolism to urea as a result of increased synthesis of arginase enzyme involved in urea production (Yanardag and Sacan, 2007). Generally, the renal damage and glomerular filtration impairment were noticed in kidney as a result of ethanol toxicity (Pari and Suresh, 2008). This may account for the increased level of serum urea, creatinine as well as lowered creatinine clearance seen in alcohol-treated rats. The excess release of liver and kidney biochemical markers could be a result of ethanol-induced membrane lipid peroxidation.

Lipid peroxidation has been implicated in a number of deleterious effects such as decreased membrane integrity, increased hemolytic and erythrocyte deformation (Meagher et al., 1999; Kasdallah-Grissa et al., 2006). In correlation with previous studies ethanol-fed rats exhibited a large amount of MDA in serum, hepatic and renal tissues. Pre-treatment of ethanol-fed rats with OFIj significantly reversed serum, hepatic and renal lipid peroxidation as evidenced by the decrease of MDA content to near control levels.

Consistent with the attenuation of lipid peroxidation, treatment of ethanol-fed rats with OFIJ was associated with a corresponding reduction in levels of serum bioche-

mical markers related to the hepatic and renal damage, indicating a protective role of OFIj against ethanol toxicity in liver. The chemical analysis of OFIj demonstrate the presence of phenolics acids such as gallic, protocatechic, 4-hydroxybenzoic, vanillic and syrengic acids and flavonoids like quercetin, luteolin, kaempferol and isorhamnetin (Alimi et al., 2012b). The OFIj active principles may acts as scavengers of free radicals and which causes the oxidative process in liver and kidney cells.

It has been shown that the main nutraceutical benefit of prickly pears and their juice has been attributed to the flavonoids contents (Kuti, 2004). It was also demonstrated that flavonoids can be incorporated in cells plasma membranes, which becomes more ordered and therefore enhances their stability (Chaudhuri et al., 2007). The localization of flavonoids in the plasma membranes could strictly hinder the diffusion of free radicals, and thereby decreases resulting damage (Dobrzynska et al., 2005). Such flavonoids proprieties could explain the increase of hepatic and renal cells membrane integrity evidenced by the decrease of MDA contents, and the reduction of serum pathology markers in alcoholic rats pre-treated with OFIj. Rather than scavenging and stabilizing capacities, it has been demonstrated that flavonoids may also inhibit the CYP 2E1 activity and/or decrease its content, thereby contributing to inhibit and/or to decrease ethanol metabolism, hence the occurrence of oxidative stress (Orellana et al., 2002).

Conclusion

The present study reported for the first time that a dietary regimen enriched with *O. ficus indica f. inermis* prickly pear juice could prevent ethanol-induced toxicity on the blood, liver and kidney in rats by avoiding anemia, the decrease of hemoglobin content, the normalization of the biochemical markers related to liver and kidney integrity and the inhibition of lipid peroxidation process. Multiple mechanisms are suggested in this study to explain the beneficial effect of OFIj, from being able to scavenge ROS and stabilizing liver and kidney membrane integrity until the possible inhibitor effect on ethanol metabolism. For this reason OFIj consumption may provide a useful approach for decreasing alcoholic damages to blood, liver and kidney functions.

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