Opuntia ficus indica f. inermis fruit juice alleviates ethanol-induced kidney injury in rats

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ABSTRACT

The present work studied the protective effect of Opuntia ficus indica f. inermis fruit juice (OFIJ) against ethanol-induced renal dysfunction in rats. Chronic ethanol administration (3 g/kg b.w) during 12 weeks to Wistar rats, significantly (p < 0.01) increased the serum and kidney malondialdehyde (MDA), the serum contents of urea, creatinine, gamma glutamyl transferase (GGT) and the urinary glucose and protein levels. Whereas, copious ethanol intoxication significantly (p < 0.01) decreased the vitamin E and C levels in serum and kidney tissue as well as the glutathione content (GSH) and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and caused a severe renal histological injuries. Rivalry pretreatment of ethanol-fed rats with OFIJ (20 and 40 ml/kg b.w., orally), interestingly attenuated the increases in serum and kidney MDA, and enhanced the antioxidant status of the rats by increasing the levels of SOD, CAT, (GSH-Px), GSH and vitamins. The biochemical findings positively corroborate with the amelioration of kidney histological morphology. The results suggest that the renal protective effect of Opuntia ficus indica f. inermis fruit juice (OFIJ) is by attenuating oxidative stress induced by copious ethanol supplement.

Keywords: Opuntia; Juice; Vitamins; Ethanol; Kidney; Toxicity; Creatinine; Urea.

1. INTRODUCTION

Alcoholic beverages are the second most used drug in the world, behind smoking. According to the World Health Organization report (2005), approximately 2 billion people worldwide consume alcohol, and about 76 million of them have been estimated to be suffering from alcohol consumption disorders [1]. Oxidative stress has been suggested as playing a central role in many pathways of alcohol-induced damage in a variety of systems, such as liver, central nervous system and kidney [2]. The metabolism of ethanol, as a major constituent of alcoholic beverages, generates acetaldehyde and reactive oxygen species (ROS), which may damages macromolecules in the cell, including lipids, proteins and DNA [3]. It has been demonstrated that chronic ethanol exposure increased ROS production, decreased antioxidant defenses and enhanced the fatty acid oxidation by kidney peroxisomes, and affected the activities of some kidney lysosomal hydrolases [4]. Oxidative stress and ROS-mediated toxicity have been implicated as the primary routes to alcohol-induced kidney injury [5]. The potential harmful effects of ROS are controlled by cellular antioxidant defense mechanisms including enzymatic defense systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), as well as non-enzymatic defense systems such as reduced glutathione (GSH), vitamins, and uric acid [6]. Synergistic and cooperative interactions between these antioxidant systems rely on the suppression of free radical formation and chain propagation. However, under certain circumstances such as chronic ethanol intake, antioxidant mechanisms fail and can bring on oxidative stress [7]. In spite of the great advances made in allopathic medicine, treatment of alcohol-induced renal injury by antioxidant drugs has not received a wide recognition. Hitherto, no effective renal...
Opuntia ficus indica f. inermis species grows throughout Tunisia and their pears are consumed as fresh fruits or used for preparing juices or jams. Due to a great number of potentially active nutrients, prickly pear and cactus juice are claimed to be health promoting food. Chemical analysis of Opuntia ficus indica f. inermis pear juice revealed the presence of polyphenols, flavonoids, ascorbic acid, carotenoids, and betalains and could protect erythrocytes from ethanol-induced erythrocyte damages in rat’s [10]. The pharmaceutical interest of Opuntia prickly pear is due to its antiulcerogenic and anti diabetic properties [11]. It was also used as adjuvant for cancer therapy [12]. So far, there is no information on the beneficial effects of this plant against alcohol-induced oxidative injury in kidneys. Therefore, this study was undertaken to evaluate the possible renal protective effect of Opuntia ficus indica f. inermis pear juice (OFIJ) in alcohol treated rats.

The present study reports whether the gastric instillation of Opuntia ficus indica f. inermis prickly pears juice (OFIJ), could prevent ethanol-induced kidney injury in Wistar rats. Thus, the serum levels of creatinine, urea, gamma glutamyl transferase (GGT) and the urinary levels of glucose and protein were determined. More so, kidney levels of malondialdehyde (MDA), reduced glutathione (GSH), and the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) were also analyzed.

2. MATERIALS AND METHODS

2.1. Chemicals

Ethanol was purchased from Carlo Erba (Reag.Ph. Eur.-Reag.USP), trichloroacetic acid (TCA), butylated hydroxytoluene (BHT), 2-thiobarbituric acid (TBA), 2,4-dinitrophenyl-hydrazine (DNPH), dithiobis-2-nitrobenzoic acid (DTNB), Folin-Ciocalteu reagent, thiourea, sulfuric acid, 2,2-dipyridyl were purchased from Sigma chemical Co. (St. Louis, MO, USA). All other used chemicals and reagents were for analytic grade.

2.2. Preparation of prickly pear juice (OFIJ)

Mature prickly pears of O. ficus indica f. inermis species (purple-skinned) were collected in the municipal area of Gafsa (Tunisia). The unpeeled fruit was washed and grounded using a Musermax double bladed mill. The resulting juice was then filtered through a colander (0.5 mm mesh size) and centrifuged at 3000 × g for 10 min to discard hard fibers. The clarified juice was then collected and stored at −21 °C until use.

2.3. Animals housing conditions

Two months old male Wistar rats (n = 40), weighing about 120 − 140 g, purchased from Pasteur institute (Tunisia), were maintained for a two weeks adaptation period under the same conditions of temperature (22 ± 2 °C), relative humidity (70 ± 4%), and a 12 h light/dark cycle. Animals were fed with commercial pellets, given tap water ad libitum and cared according to the Tunisian code of practice for the Care and Use of Animals for Scientific Purposes.

2.4. Experimental design

After the adaptation period, animals were divided into five experimental groups (n = 8) and treated for 12 weeks with two consecutive intra-gastric intubations of vehicle (distilled water), OFIJ, ethanol (prepared at 30% in distilled water which was equivalent to 3 g/kg b.w.) or both OFIJ and ethanol according to the scheme described in Table 1.

2.5 Preparation of samples

In the last week of the experimental period urine free of food and faeces was daily collected into ice-cold glass tubes for the determination of protein and glucose levels. At the end of experimental period animals were sacrificed by cervical decapitation under light ether anesthesia. The serum was collected by centri fugation of the whole blood at 1000 × g for 10 min at 4 °C and stored at −80 °C until analysis. Kidney samples were quickly removed, washed in ice-cold 1.15% KCl solution, homogenized into 2 ml ice-cold lyses buffer (pH 7.4) and centrifuged for 20 min at 3000 × g, 4 °C. The collected supernatants were stored at −80 °C until the analysis.

2.6 Biochemical assays

The serum creatinine, urea and GGT activities as well urinary glucose level were assayed spectrophotometrically using BioMaghreb diagnostic kits. In serum and kidney homogenate the level of lipid peroxidation was measured as malondialdehyde content (MDA) according to the method of Okawa et al. (1979) [13]. In kidney homogenate total GSH contents were measured by Ellman’s reaction using 5,5-dithiobis 2-nitrobenzoic acid according to the method of Moron et al. (1979) [14]. SOD activity was estimated according to the method described by Misra and Fridovich (1972) [15]. CAT activity was determined by measuring hydrogen peroxide decomposition at 240 nm according to the method described by Aebi (1984) [16]. GSH-Px activity was assayed using the method described by Flohe and Gunzler (1984) [17], by the subsequent oxidation of NADPH at 240 nm. In serum and kidney homogenate the levels of ascorbic acid (vitamin C) concentration was measured according to the method of Omaye et al., (1979) [18] more so vitamin E...
level was determined based on the method of Desai (1984) [19].

2.7. Protein determination
Serum, urinary and kidney homogenate protein contents were determined according to Lowry’s method using bovine serum albumin as standard [20].

2.8. Kidney histopathological studies
Kidney tissues were cut into about 5-cm-thick slices and fixed with 10% phosphate-buffered formalin (pH 7.4). The tissue slices were dehydrated through ascending grades of alcohol, and embedded in paraffin. Tissue sections of 5–8 μm were made using microtome, and stained with hematoxylin-eosin solutions (H&E). Tissue preparations were observed and micro-photographed under a light BH2 Olympus microscope.

2.9. Statistical analysis
All the values have been reported as means ± standard deviation of triplicate samples and were analyzed statistically by one way ANOVA and different group means were compared by Duncan’s multiple range tests; p < 0.05 was considered significant in all cases. The software SPSS 12.0 was used for analysis of data.

3. RESULTS
Following the exposure of experimental groups; the effects of alcohol and the Opuntia ficus indica f. inermis pear juice (OFIJ) supplementations on kidney damages index and anti-oxidative role were evaluated.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Volumes of treatments (ml/kg b.w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9 a.m.</td>
</tr>
<tr>
<td>Control</td>
<td>Water (10)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>Water (10)</td>
</tr>
<tr>
<td>OFIJ2+Ethanol</td>
<td>OFIJ (20)</td>
</tr>
<tr>
<td>OFIJ4+Ethanol</td>
<td>OFIJ (40)</td>
</tr>
<tr>
<td>OFIJ4</td>
<td>OFIJ (40)</td>
</tr>
</tbody>
</table>

Distilled water was used as vehicle (Water). OFIJ2 and OFIJ4, Opuntia ficus indica f. inermis prickly pears juice respectively given at 20 and 40 ml/kg b.w. doses.

Table 1. Scheme of drugs treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Creatinie¹</td>
</tr>
<tr>
<td>Control</td>
<td>0.42 ± 0.01¹d</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.76 ± 0.09¹0</td>
</tr>
<tr>
<td>OFIJ2+Ethanol</td>
<td>0.53 ± 0.06²0</td>
</tr>
<tr>
<td>OFIJ4+Ethanol</td>
<td>0.46 ± 0.03³⁰</td>
</tr>
<tr>
<td>OFIJ4</td>
<td>0.41±0.02⁴</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD, for eight rats in each group. ¹mg/dl, ²IU/l. OFIJ2 and OFIJ4: Opuntia ficus indica f. inermis pear juice at doses 20 and 40 ml/kg b.w., respectively.

Table 2. Effects of ethanol and OFIJ treatments on serum creatinin, urea and GGT contents of the control and experimental rats

Table 2, shows that the exposure of Wistar rats to chronic ethanol intoxication (twelve weeks), significantly (p < 0.01) increased serum creatinine and urea levels as well the activity of GGT when compared with control rats. Whereas pre-treatment of ethanol-fed rats with low (OFIJ2) and high (OFIJ4) doses of OFIJ significantly reversed (p < 0.01) the ethanol-induced increase in serum creatinine and urea levels as well the activity of GGT when compared with ethanol group. In rats only received a high OFIJ doses (OFIJ4) a slight reduction in serum creatinine, urea and GGT levels was observed when compared with control rats.

Table 3 showed that chronic ethanol administration was found to cause a significant decrease (p < 0.01) in the levels of serum and kidney vitamin E, vitamin C and a significant increase (p < 0.01) in the level of malondialdehyde (MDA) content when compared with the control group. However OFIJ supplement in low (OFIJ2) and high (OFIJ4) doses significantly (p < 0.05) increased vitamin E and vitamin C levels and decreased the MDA production in dose-dependent manner when compared with ethanol group. The administration of a high dose of OFIJ alone for intact rats slightly increased the levels of serum and kidney vitamin E, vitamin C and slightly reduced the lipid peroxidation product (MDA) when compared with ethanol-fed rats.

Table 4 represents the renal GSH level and the activities of antioxidant enzymes namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase. Chronic ethanol administration was found to cause a significant decrease (p < 0.01) in renal GSH level and the activities of antioxidant enzymes when compared with control group. However OFIJ supplement in low (OFIJ2) and high (OFIJ4) doses significantly (p < 0.01) increased kidney SOD, CAT, GSH-Px activities and GSH level in dose-dependent manner when compared with ethanol group. The administration of a high dose of OFIJ alone for intact rats slightly increased the level of GSH, the SOD and CAT activities compared to control group, whereas GSH-Px activity appeared normal.

Figure 1. Effects of ethanol and OFIJ treatments on serum protein and glucose levels. Values are expressed as means ± SD (n = 8). ² p < 0.01, ²² p < 0.01 versus ethanol group; ³ p < 0.05, ³³ p < 0.01 versus control group. OFIJ2 and OFIJ4: Opuntia ficus indica f. inermis pear juice at doses 20 and 40 ml/kg b.w., respectively.
Ethanol treatment significantly (p < 0.01) increased urinary glucose and protein levels respectively by 61 and 176%. However, treatment with OFIJ in low (OFIJ2) and high (OFIJ4) doses significantly reduced (p < 0.01) glucose level respectively by 16.32 % and 38% and protein level respectively by 26.78 % and 37.14% when compared with ethanol group. Treatment of intact rats with a high dose of OFIJ (OFIJ4) reduced urinary glucose and protein levels respectively by 2.32% and 2.78 %when compared with control group.

Figure 2 some of the histological observations in slides from ethanol-treated rats include tubular and renal cells necrosis as well the congestion of blood vessels was observed. These histological alterations were significantly reduced in slides obtained from rats treated with ethanol and OFIJ (20 and 40 ml/kg b.w.). No visible lesions were observed in rats treated with OFIJ4 alone. The results of the histopathology correlated with the biochemical changes in the serum and urinary indices of the animals.

Table 3. Effects of ethanol and OFIJ treatments on vitamin E, vitamin C and MDA contents in serum and kidney of the control and experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Vitamin E</th>
<th>Vitamin C</th>
<th>MDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Kidney</td>
<td>Serum</td>
</tr>
<tr>
<td>Control</td>
<td>2.3 ± 0.42</td>
<td>18.34 ± 0.83</td>
<td>2.34±0.12</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.79 ± 0.18</td>
<td>11.3 ± 0.73</td>
<td>0.96±0.02</td>
</tr>
<tr>
<td>OFIJ2+Ethanol</td>
<td>1.87 ± 0.04</td>
<td>16.72 ± 0.91</td>
<td>1.83±0.08</td>
</tr>
<tr>
<td>OFIJ4+Ethanol</td>
<td>2.21 ± 0.32</td>
<td>18.13±0.41</td>
<td>2.29±0.06</td>
</tr>
<tr>
<td>OFIJ4</td>
<td>2.37±0.11</td>
<td>19.74±0.33</td>
<td>2.79±0.02</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD, for eight rats in each group. * p < 0.01 when compared with control group; ** p < 0.05, *** p < 0.01 when compared with control group. μg/mg tissue and nmol/mg protein.

Table 4. Effects of ethanol and OFIJ treatments on renal GSH content and the activities of antioxidant enzymes.

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH⁺</th>
<th>SOD⁻</th>
<th>CAT⁺</th>
<th>GSH-Px⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.21±0.04</td>
<td>78.12 ± 0.43</td>
<td>21.3±0.6</td>
<td>22.4±0.8</td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.11±0.02</td>
<td>51.6 ± 0.91</td>
<td>15.61±0.2</td>
<td>14.09±1.2</td>
</tr>
<tr>
<td>OFIJ2+Ethanol</td>
<td>0.17±0.06</td>
<td>69.81±1.2</td>
<td>17.8±0.4</td>
<td>19.51±0.7</td>
</tr>
<tr>
<td>OFIJ4+Ethanol</td>
<td>0.20±0.08</td>
<td>76.53±0.5</td>
<td>20.49±0.1</td>
<td>21.3±0.6</td>
</tr>
<tr>
<td>OFIJ4</td>
<td>0.24±0.06</td>
<td>79.64±0.2</td>
<td>22.07±0.7</td>
<td>22.42±0.3</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD, for eight rats in each group. * p < 0.01 when compared with ethanol group; ** p < 0.05, *** p < 0.01 when compared with control group. μmol GSH/mg protein,

4. DISCUSSION

Oxidative stress is gradually more suspected to the initiation and progression of diseases, including those caused by chronic ethanol exposure. As an intensive oxidative stressor and hyperosmotic micromolecule, ethanol produces excessive acetaldehyde and reactive oxygen species (ROS) during metabolism and results in lipid peroxidation, protein oxidation, DNA damage and adduct formation by which it induces injury to kidney and other organs [21]. Cellular systems are protected from ROS by an array of defenses composed of various antioxidants. However copious ethanol intake raises ROS production which could overpower the defense systems and causes kidney injury. The present study shows that chronic ethanol administration to Wistar rats, for twelve
consecutive weeks, caused a significant ($p < 0.01$) increase of urinary glucose and protein levels. Our results are in agreement with those reported by Adaramoye and Aluko (2011) [2]. The increase of glycosuria and proteinuria suggests that ethanol abuse may result in a generalized reduction in the proximal tubular cells reabsorptive ability. This hypothesis is supported by previous study indicating that ethanol interferes with the carrier functions of these cells by decreasing Na+/K+−ATPase activity [22]. When OFIJ was administered to ethanol-fed rats the urinary protein and glucose levels was significantly reduced. This shows that OFIJ could preserve the renal structural integrity from the adverse effects of ethanol.

It is also well known that serum creatinine and urea levels as well as the GGT activity are consistent biochemical indices for renal function and their increases indicate the kidney function impairment such as acute glomerulonephritis [23]. In the present study, it is note worthy that chronic ethanol intoxication for 12 weeks caused significant elevation of serum urea and creatinine levels as well as GGT activity. This observation has been also reported by Cigremis et al (2004) [6]. These increases could be linked to adverse effect of ethanol, which results in the decline of the glomerular filtration rate. The fact that these parameters were reversed to near normal following OFIJ treatment further confirmed the protective effect of these natural products against ethanol-induced renal dysfunction.

The histopathological study shows that chronic ethanol intake induced the tubular and renal cells necrosis as well the congestion of blood vessels. In the present study we showed that OFIJ administration diminished the ethanol-induced kidney tissue injury as monitored by normal kidney architecture when compared with alcoholic rats. The OFIJ-improvement of ethanol-fed rats kidney tissues appear in correlation with the corresponding reduction of urinary glucose and protein levels as well as the levels of serum urea, creatinine and the leakage of GGT to the blood stream.

It was obvious from our results that chronic ethanol exposure significantly ($p < 0.01$) increased the MDA concentration in the serum and kidney tissue and attenuates the levels of vitamins E and C. Studies have shown that ethanol consumption may result in increased oxidative stress by the ROS superoxide overproduction which can later initiate free radicals chain reactions of lipid peroxidation [24]. As ROS scavengers, vitamins E and C has been shown to be effective against superoxide radical, hydrogen peroxide, hydroxyl radical and singlet oxygen [25]. The observed decrease in the levels of serum and kidney vitamin E and C in ethanol-fed rats could be the results of increased uses of these antioxidants in scavenging the ethanol-overproduced free radicals. In the present study treatment of ethanol-intoxicated rats with OFIJ increased the levels of vitamin E and C. This may be due to the effect of OFIJ antioxidant compounds on the ROS, produced during ethanol metabolism. Thus, OFIJ may exert a beneficial effect in neutralizing the toxic free radicals in kidney.

In normal conditions kidney possess a powerful antioxidant defense system, including enzymatic and non-enzymatic antioxidants such as SOD, CAT, GSH-Px and GSH [31]. However, under certain circumstances, chronic ethanol intake and antioxidant mechanisms fail can bring on oxidative stress [7]. In consistent with these reports, our results also showed that chronic ethanol administration to untreated rats, decreased the kidney GSH, SOD, CAT and GSH-Px levels. Such decreases can result from ROS depletion of the above antioxidants or to the suppression of the de novo protein synthesis [26]. We have previously demonstrated that OFIJ appears as a good candidate in the prevention of ethanol-induced antioxidant status impairment in rat’s erythrocytes and liver [10, 27]. In the present study we showed that OFIJ diminished the ethanol-induced the renal tissue injury as monitored by normal kidney architecture when compared with alcoholic rats. Further more renal GSH content and antioxidant enzymes activities, namely, SOD, CAT and GSH-Px were visibly ameliorated when OFIJ was supplemented to ethanol-fed rat’s diet.

A wide range of natural antioxidant compounds can protect the kidney from damage via a variety of mechanisms. In our previous study we have showed that OFIJ was rich in polyphenols, flavonoids, ascorbic acid, carotenoids, and betalains. We have also demonstrated that OFIJ supplement increased plasma scavenging activity of control and ethanol fed rats [10]. The OFIJ active principles may acts by scavenging free radicals and ROS that causes the oxidative process in kidney cells. The restoration of kidney antioxidant status when OFIJ was supplemented to alcoholic rat’s diet may be attributed, in part, to the antioxidant sparing action of the phenolic compounds. In addition, administration of high OFIJ dose to untreated rats slightly enhanced production of GSH, SOD, CAT and GSH-Px. Hence, renal protective effect of OFIJ could be attributed in part to its safeguard and/or inductive effects on GSH, SOD, CAT and GSH-Px synthesis. Our results are in agreements with those of Moskaug et al. (2005) [28] which reported that phenolic compounds were able to induce GSH biosynthesis via the up-regulation of γ-glutamylcysteine synthetase expression.

The HPLC analysis of OFIJ revealed the presence of phenolic acids, such as gallic, protocatechatic, 4-hydroxybenzoic, vanillic and syrengic acids as well as flavonoids compounds like quercetin, luteolin, kaempferol and isorhamnetin [29]. This kind of antioxidants was able
to liberate a hydrogen proton from their hydroxyl groups which could scavenge free radicals and prevent renal cells from oxidative damage. It was also demonstrated that flavonoids can be incorporated in cell plasma membranes, which becomes more ordered and therefore enhances their stability [30]. The localization of flavonoids in the plasma membranes could strictly hinder the diffusion of free radicals, and thereby decreases resulting damage. Such flavonoids properties could explain the increase of renal cells membrane integrity evidenced by the decrease of MDA contents, the restoration of the histological morphology, and the reduction of the renal GGT leakage to the blood stream in alcoholic rats. 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 [6]. I our previous study we have also showed the presence of ascorbic acid, carotenoids and betalains in OFJ [10]. These kinds of antioxidant are also known for their scavenging and reducing activities [8], and their supplement through OFJ administration to ethanol-fed rats could preserve/restore the E and C vitamins stock and contributes to the protective effect of OFIJ.

5. CONCLUSION

The present report is the first study examining the effect of OFJ on ethanol-inducing kidney injury. The active components of OFIJ could act synergistically in preventing ethanol toxicity, by scavenging free radicals and ROS, by stabilizing renal cells membrane integrity and restoring antioxidant enzymes levels. Hence, our study suggests that OFJI consumption may provide a useful approach for decreasing ethanol-induced kidney damage.

6. ACKNOWLEDGMENTS

This research was funded by the Tunisian Ministry of Higher Education and Scientific Research through Research Unit of Macromolecular Biochemistry and Genetics, Faculty of Sciences of Gafsa and Integrated Physiology Laboratory, Faculty of Science of Bizerte.

7. REFERENCES


Cite this article as: