INTRODUCTION

Alcohol is the most commonly used beverage worldwide and is considered a socially acceptable toxin. At present, except for the control of alcohol abuse, there are no effective modalities of either prevention or treatment alcoholism is one of the largest health problems in the United States [1]. There are approximately 10 million alcoholics in America and alcoholism alone leads to over 2,000,000 deaths per year, requiring over 12 million dollars annually in medical expenditures [2]. Alcohol-related morbidity and mortality are due to alcohol-induced liver disease (ALD [directly]), periodontitis, and cancer (indirectly), as the association between chronic alcoholism and cirrhosis of liver is a well-established clinical manifestation [3].

It is now well accepted that the progression of liver injury consequent to chronic alcohol abuse is a multifactorial event that involves a number of genetic and environmental factors. Among these factors, there is a growing interest in the role of free radical-mediated oxidative stress. The involvement of oxidative injury in ethanol toxicity was first proposed in the early 1960s and subsequently supported by a number of experimental studies showing that ethanol promotes the formation of a variety of free radical intermediates (oxygen-derived radicals, 1-hydroxyethyl radicals (CH3C•OH; HER), NO, lipid-derived radicals) by several cell types, including hepatocytes, kuffer cells, endothelial cells, and infiltrating inflammatory leukocytes. However, the most convincing evidence to clarify the role of oxidative damage in the pathogenesis of alcohol-induced liver injury has been obtained using a procedure in which alcohol is fed enterally to rodents [4]. The rats fed ethanol in combination with diets rich in unsaturated fatty acids from maize or fish oils develop liver damage. This outcome is associated with an increase in the hepatic content of lipid peroxidation (LPO) products [5], suggesting that the generation of aldehydeic end products of the peroxidative breakdown of PUFA promotes ethanol toxicity. The relevance of these observations to human ALD is demonstrated by several clinical studies showing that serum markers of LPO, such as conjugated dienes, malondialdehyde (MDA), 4-hydroxynonenal, and F2-isoprostanes, are increased in patients with ALD [6]. Moreover, immunohistochemical analysis of liver biopsies from patients with alcoholism reveals that proteins adducted by LPO products are localized in the areas of liver fatty infiltration, focal necrosis, and fibrosis [7].

Ethanol or its metabolites can prompt a sharp increase of free radicals in the human body (e.g., hepatic cells in liver) by acting as a prooxidant or by reducing antioxidant levels and contributing to the progression of a variety of chronic diseases [8]. Ethanol intake increases free radicals or reactive oxygen species (ROS) production and causes oxidative stress by compromising the antioxidant defense system. ROS are highly reactive and can damage lipids, proteins, and DNA [9]. Antioxidants are the substances that when present in low concentration significantly delay or reduce the oxidation of the substrate [10]. A wide variety of plants and their active principles have undergone experimental studies in diverse models of liver and kidney injuries. Recently, there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants and has gained an immense importance,
and current research trends are directed toward finding naturally occurring antioxidants particularly of plant origin in reducing such free radical induced tissue injury [11]. Many plant species have been investigated in search of natural antioxidants and novel antioxidants, but generally there is still demand to find more information concerning the antioxidant potential of plant species. It has been mentioned that the antioxidant activity of plants might be due to their phenolic compounds [12].

Natural bioactive compounds found in foods offer a more appealing therapeutic option for many chronic diseases. In particular, fruits and vegetables rich in phytochemicals have been investigated for their potential health benefits [13]. Among these fruits, mango (Mangifera indica L.) provides a number of well-known bioactive compounds such as carotenoids, vitamin C and phenolic compounds that have been shown to exhibit both antioxidant and anti-inflammatory properties [14]. For example, the aqueous extract of the stem bark and leaves of mango was shown to effectively lower blood glucose in streptozotocin-induced diabetic rats, and mango stem bark extract has been demonstrated to exhibit anti-inflammatory properties by inhibiting egg albumin induced paw edema in rats [15]. Furthermore, mango juice has been shown to inhibit free radical production and neoplastic transformation in BALB/3T3 and HL-60 cells, demonstrating potent antioxidant and anti-cancer properties [16]. Studies focused on polyphenolic components of the mango fruit, quercetin, and the aglycone derivative of mangiferin (i.e., norathyriol), have revealed that these components inhibit the activation of all three isoforms of peroxisome proliferator-activated receptor [17].

Because oxidative damage is an important mechanism by which alcohol induces cell damage, in this study, we want to explore the possibility of antioxidant compounds present in mango juice (non-irradiated and γ-irradiated [NMJ and GMJ]) could protect and reduce the effects of oxidative damage on liver and kidney tissues.

METHODS

Chemicals

All chemicals and solvents used in this study were of analytical grade and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher Scientific (Pittsburg, PA, USA), Merck (Mumbai, Maharashtra, India), Himedia (Mumbai, Maharashtra, India), and Qualigens (Mumbai, Maharashtra, India). The water used in the analysis was obtained from a Milli-Q water purification system manufactured by Millipore (Bedford, MA, USA). All solvents used as the mobile phase were previously filtered through 0.45 μm membranes (Millipore) and degassed before use.

Standard phenolic compounds and antioxidants

The standard phenolic compounds; gallic acid, protocatechuic acid, p-OH-benzoic acid, vanillic acid, syringic acid, ellagic acid, caffeic acid, p-coumaric acid, m-coumaric acid, ferulic acid, synapic acid, rutin, and quercetin were purchased from Sigma-Aldrich (Steinheim, Germany) and (+)-catechin and chlorogenic acid were supplied from Fluka (Buchs, Switzerland). Stock solutions of all the phenolic standards were prepared in methanol.

The standard antioxidants used in this study were ascorbic acid, butylated hydroxyl anisole (BHA), and butylated hydroxyl toluene (BHT). Ascorbic acid was dissolved in double distilled water. BHT and BHA were dissolved in methanol.

Procurement and maintenance of experimental animals

Male Albino Wistar rats (n=36) obtained from Sri Raghavendra Suppliers, Bangalore, Karnataka, India aged 6 months and weighing 180±20 g were used in this study. The rats were provided with standard pellet diet (Lipton rat feed Ltd., Pune, Maharashtra, India) and water ad libitum throughout the experimental period. They were housed in a clean, dry polypropylene cages and maintained in a well-ventilated animal house with 12 h light-12 h dark cycle, as per the guidelines of the National Institute of Nutrition, Indian Council for Medical Research, Hyderabad, India and approved by the Animals Ethical Committee, Sri Venkateswara University (Resolution No. 39/2012-2013/1/a/CPCSEA/IAEC/SVU/OVS-KN/Dt.08-07-2012).

Preparation of mango juice and γ-irradiation

Fresh, healthy, and ripe mango fruits var. Alphonso were procured from the local market, Tirupati, Andhra Pradesh, South India and then processed according to Naresh et al. [14]. The obtained mango juice was grouped into two lots according to the dose of irradiation received 0 (non-irradiated) and 1 kGy in 500 mL air tight containers each. The irradiation was carried out at ambient temperature (26±2°C) in a cobalt-60 irradiator (model GC-5000, Board of Radiation and Isotope Technology, Mumbai, Maharashtra, India; dose rate of 5.5 kGy/h) at Food Technology Division, Bhabha Atomic Research Centre, Mumbai, Maharashtra, India. Both irradiated and NMJ samples were stored at −20°C for no longer than 2 months.

Extraction and characterization of mango juice polyphenols by high-performance liquid chromatography (HPLC)

Both NMJ and GMJ (50 mL) samples were adjusted to pH 7.0 with 2 N NaOH and extracted with 50 mL ethyl acetate at 30°C by stirring for 5 minutes, using a casing vessel with a reflux condenser. The mixture was then centrifuged at 5000 rpm for 5 minutes. The organic layer was transferred, and the extraction process was repeated twice with 50 mL ethyl acetate. The three organic layers were pooled and evaporated to dryness at 35°C by means of a helical gas flow (nitrogen) at 1.8 bar by vortexing action. The resulting residue was redisolved in 2 mL methanol.

Individual polyphenolic compounds were characterized and quantified by HPLC system (Model PU 980; JASCO International Co. Ltd., Tokyo, Japan), equipped with a C18 reverse phase stainless steel column (250 mm×4.6 mm, Thermoa Hypersil-Keystone; Thermo Fisher Scientific Inc., Waltham, MA, USA), which was kept at 25°C with a Photodiode Array detector as previously described by Naresh et al. [14].

Antioxidant screening of mango juice

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of mango juice was determined by the method of Halliwell et al. [18] with slight modifications. The reaction mixture consisted of 1 mM EDTA, 10 mM FeCl3, 10 mM H2O2, 10 mM deoxyribose, 1 mL of different dilutions of the mango juice (50-250 μL), 50 mM phosphate buffer (pH 7.4), and 0.1 mL of ascorbic acid in sequence. The mixture was incubated at 37°C for 1 hr. 1 mL portion of the incubated mixture was mixed with 1 mL of 10% trichloroacetic acid and 1 mL of 0.5% thiobarbituric acid (TBA) (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm.

Scavenging of hydrogen peroxide

The ability of mango juice to scavenge hydrogen peroxide (H2O2) was determined according to the method of Ruch et al. [19] with slight modifications. A solution of H2O2 (2 mM) was prepared in phosphate buffer (0.1 mM, pH 7.4) at 20°C. An aliquot of the mango juice was dissolved in phosphate buffer (1 mL; 0.1 mM, pH 7.4) at various concentrations (50-250 μL) and mixed with 600 μL of H2O2. BHT and ascorbic acid were used as the reference compounds. The concentration of H2O2 was measured by reading the absorbance at 230 nm after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The mango juice samples were capable of scavenging hydrogen peroxide in a concentration-dependent manner.

Superoxide anion scavenging activity

The superoxide anion (O2•−; superoxide) scavenging activity of mango juice was determined by the method of Nishikimi et al. [20] with slight modifications. The superoxide anion was generated using two
non-enzymatic systems. The first consisted of 6.30 μM of nitro blue tetrazolium (NBT), 30 μM of phenazine methosulfate and 156 μM nicotine adenine dinucleotide in 0.1 M phosphate buffer, pH 7.4. The product of the reduction of NBT was followed spectrophotometrically at 560 nm as an index of the superoxide anion production.

**Treatment protocol**

The rats were divided into 6 groups of six animals each, and the treatment was given every day via orogastric tube for 30 days.

- **Group I (n=6):** Rats were given normal pellet diet without any treatment and served as normal control (NC) group.
- **Group II (n=6):** Rats received absolute ethanol orally at the dose of 2 g/kg via orogastric tube for 30 days and served as EtOH group.
- **Group III (n=6):** Rats received oral administration of 3 ml/kg non-irradiated mango juice and served as NMJ group.
- **Group IV (n=6):** Rats received oral administration of 3 ml/kg γ-irradiated mango juice and served as GMJ group.
- **Group V (n=6):** Rats received oral administration of 2 g/kg ethanol and 3 ml/kg non-irradiated mango juice and served as EtOH+NMJ group.
- **Group VI (n=6):** Rats received oral administration of 2 g/kg ethanol and 3 ml/kg γ-irradiated mango juice and served as EtOH+GMJ group.

**Collection of blood and tissues**

At the end of experimental period, the animals were fasted for 12 hrs to minimize dietary changes and sacrificed by cervical dislocation. Immediately blood was collected into sterile tubes by cardiac puncture and serum was separated from the cells by centrifugation at 3000 rpm for 10 minutes at 4°C and stored at ~80°C until analysis. The tissues (liver and kidney) were removed and washed thoroughly with ice-cold saline (0.9 % NaCl) and processed appropriately, stored at ~20°C in propylene containers until assays were carried out.

**Preparation of the tissue homogenate**

Samples of liver and kidney (100 mg/mL) tissues were slightly thawed, minced with scissors, and homogenized in 50 mM Tri-HCl buffer (pH 8.0) containing 0.25 M sucrose and 1mM PMSF using a Potter Elvejhem homogenizer. The homogenate was passed through two layers of cheesecloth to remove fat, and the filtrate was centrifuged at 30,000 × g on high speed refrigerated centrifuge for 30 minutes. The resulted supernatant was used for assaying the various biochemical parameters.

**Histopathological studies**

Histopathological parameters were studied at the Department of Pathology, S. V. Veterinary University, Tirupati, Andhra Pradesh, India. The liver and kidney tissues were fixed for 48 hrs in 4% paraformaldehyde, were washed, dehydrated with alcohol, cleared with xylene and paraaffin blocks were made. Serial sections of 5 μm thickness were cut using a rotary microtome. The sections were then deparaffinized with xylene and hydrated in descending grades of alcohol. The slides were then transferred to hematoxylin for 10 minutes, followed by rinsing with water, differentiated in 1% acid alcohol, rinsed in water, bluing in running tap water or 1 % lithium carbonate. Later counterstained with eosin, rinsed with water, dehydrated with ascending grades of alcohol, cleared with xylene, and mounted using neutral (DPX) mountant on glass slides. These slides were examined using light microscope.

**Biochemical estimations**

**Liver function test**

Colorimetric determination of alanine aminotransferase (ALT) or aspartate aminotransferase (AST) was estimated by measuring the amount of pyruvate or oxaloacetate produced by forming 2,4-dinitrophenylhydrazine according to the method of Reitman and Frankel [21]. The color of which was measured at 546 nm. Gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase (ALP) were assayed using kits provided from Span Diagnostics Ltd., Surat, Gujarat, India.

**Kidney function test**

Uric acid (UA), blood nitrogen urea, and serum creatinine (Cr) were assayed in serum using kits provided from Span Diagnostics Ltd., Surat, Gujarat, India.

**Determination of MDA**

The extent of LPO was estimated as the concentration of TBA-reactive product MDA. It was assayed colorimetrically in liver and kidney homogenates according to the method of Ohkawa et al [22]. MDA was determined using 1 ml of trichloroacetic acid 10% and 1 ml of TBA 0.67% and were then heated in a boiling water bath for 30 minutes. TBA-reactive substances (TBA-RS) were determined by the absorbance at 535 nm and expressed as MDA formed.

**Estimation of non-enzymatic antioxidants in tissues**

The hepatic and renal reduced glutathione (GSH) level were determined by the method of Ellman [23]. The method based on the reduction of Ellman’s reagent (5,5’-dithiobis[2-nitrobenzoic acid]) with GSH to produce a yellow compound. The reduced chromogen was directly proportional to GSH concentration, and its absorbance can be measured at 405 nm. Vitamin C was estimated by the method of Omaya et al. [24]. Ascorbic acid is oxidized by copper to form dehydroascorbic acid which reacts with 2,4-dinitrophenyl hydrazine; this undergoes further rearrangement to form a product with an absorption maximum at 520 nm.

**Assay of enzymatic antioxidants in tissues**

The hepatic and renal superoxide dismutase (SOD) activity was assayed by the method of Misra and Fridovich [25] at 480 nm for 4 min on a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 U/mg of protein. The catalase (CAT) activity was determined by the method of Aebi [26], and the absorbance of the sample was measured at 240 nm for 1 min in a UV-spectrophotometer. The activity of glutathione peroxidase (GPx) was determined by the method of Flohé and Günzler [27] in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) and the absorbance was measured at 340 nm using cumene hydrogen peroxide. The glutathione reductase (GR) enzyme activity was determined according to the method of Carlborg and Mannervik [28]. Glutathione-S-transferase (GST) activity was determined by measuring the increase in absorbance at 340 nm using 1-chloro-2, 4-dinitro benzene as a substrate by the method of Habig et al. [29]. All enzyme activities were expressed per mg protein, and the tissue protein was estimated according to the method of Lowry et al. [30] using bovine serum albumin as the standard.

**Statistical analysis**

Values are expressed as mean ± standard deviation from six animals. All the statistical analyses were carried out using the SPSS statistical tool (SPSS for windows, release 17.0.1, 2008, SPSS Inc., Chicago, IL, USA). The Duncan’s multiple range test (DMRT) and one-way analysis of variance were used to assess the differences. p<0.05 was considered as statistically significant.

**RESULTS AND DISCUSSION**

**Polyphenolic composition of mango juice**

The HPLC profile of polyphenolic compounds identified in both the control and GMJ (1 kGy) is shown in Fig. 1. The major polyphenolic compounds identified in the mango juice consist of different classes such as phenolic acids and flavonoids. There were significant differences noted in the concentration of polyphenolic compounds between non-irradiated and γ-irradiated juice samples (Table 1). γ-irradiation significantly altered, either decreased or increased the concentration of the polyphenolic compounds studied. The content of the most phenolic acids was significantly increased in 1 kGy irradiated mango juice samples, except ferulic and synapic acids, where their contents were significantly decreased by 2.2 and 2.6 folds respectively in irradiated juice samples. A similar increase in gallic acid is oxidized by copper to form dehydroascorbic acid which reacts with 2,4-dinitrophenyl hydrazine; this undergoes further rearrangement to form a product with an absorption maximum at 520 nm.
Table 1: Effects of γ-irradiation on polyphenolic compounds of mango juice (Cv. Alphonso)

<table>
<thead>
<tr>
<th>Phenolic compound</th>
<th>RT (min)</th>
<th>Irradiation dose</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 kGy</td>
<td>1 kGy</td>
<td></td>
</tr>
<tr>
<td>Gallic acid</td>
<td>5.11</td>
<td>106.3±5.3</td>
<td>376.1±9.9***</td>
</tr>
<tr>
<td>Protocatechuic acid</td>
<td>7.45</td>
<td>17.4±0.9</td>
<td>29.7±1.2**</td>
</tr>
<tr>
<td>p-OH-benzoic acid</td>
<td>11.89</td>
<td>24.3±0.8</td>
<td>43.1±1.3***</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>15.01</td>
<td>14.7±0.7</td>
<td>30.3±1.2***</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>15.34</td>
<td>20.1±0.1</td>
<td>40.7±1.3***</td>
</tr>
<tr>
<td>Syringic acid</td>
<td>16.77</td>
<td>3.4±0.3</td>
<td>13.5±0.5***</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>17.16</td>
<td>34.5±1.1</td>
<td>77.2±3.1***</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>24.41</td>
<td>105.3±5.2</td>
<td>142.3±6.4**</td>
</tr>
<tr>
<td>m-Coumaric acid</td>
<td>26.26</td>
<td>76.7±4.6</td>
<td>82.6±5.0*</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>28.33</td>
<td>40.3±1.1</td>
<td>18.4±0.9***</td>
</tr>
<tr>
<td>Sympatic acid</td>
<td>29.25</td>
<td>35.4±0.9</td>
<td>13.7±0.6***</td>
</tr>
<tr>
<td>Ellagic acid</td>
<td>33.91</td>
<td>9.3±0.5</td>
<td>21.1±1.0***</td>
</tr>
<tr>
<td>Rutin</td>
<td>34.54</td>
<td>6.4±0.6</td>
<td>12.4±0.4*</td>
</tr>
<tr>
<td>(+)-Catechin</td>
<td>37.11</td>
<td>6.0±0.3</td>
<td>12.2±0.3*</td>
</tr>
<tr>
<td>Quercetin</td>
<td>51.83</td>
<td>9.4±0.4</td>
<td>9.6±0.3†</td>
</tr>
</tbody>
</table>

RT: Retention time. ****/***/***/* significant when compared to that of control (non-irradiated) at p<0.0001, 0.001 and 0.01 respectively.
† Not significant (p>0.05); ‡ minus (−) value indicate % decrease.

A significant increase in the content of gallic, chlorogenic, p-coumaric acids, rutin, and quercetin as a result of γ-irradiation also reported in two types of Malaysian honey [32]. The increase in phenolic acids could be due to the higher extractability as a result of depolymerization and dissolution of cell wall polysaccharides during γ-irradiation [33]. There was also a significant increase in the content of flavonoids such as rutin and catechin and no increase in the content of quercetin in 1 kGy irradiated mango juice sample. The main phenolic compounds of mango pulp are gallic acid, mangiferin, quercetin-glycosides and many identified and uncharacterized hydrolyzable tannins, called gallotannins [34]. In the present study, an increase in some of the phenolic compounds observed in γ-irradiated samples might be due to the degradation of hydrolyzable tannins present in the mango juice samples. Further, irradiation exerts its effects as direct and indirect mechanisms; in case of indirect mechanism, radiolysis of water results in the production of radicals such as hydrated electrons, hydroxyl radicals, and hydrogen atoms [32]. These radicals may break the glycosidic bonds of phenolic compounds that are present in mango juice, leading to the formation of new compounds. Thus, increase in phenolic compounds in GMJ could be attributed to the release of phenolic compounds from glycosidic components and the degradation of the larger phenolic compounds into smaller ones by γ-irradiation [35].

Free radical scavenging activity of mango juice

The use of free radical scavenging assay provides an easy, rapid, and convenient method to evaluate the antioxidants and radical scavengers. Therefore, in this study, NMJ and GMJ samples were screened for their possible antioxidant and free radical scavenging activity, which served as a significant indicator of its potential antioxidant activity. The free radical scavenging effect of NMJ and GMJ, assessed using hydroxyl radical, hydrogen peroxide, superoxide anion is shown in Fig. 2. Both mango juice samples demonstrated remarkable free radical scavenging effects with higher activity when compared with standard antioxidants (Fig. 2).

Effect of ethanol and mango juice on hepatic and renal function markers

Measurement of enzymatic activities of aminotransferases (AST and ALT), ALP and GGT, is of clinical and toxicological importance, as changes in their activities are indicative of tissue damage by toxicants or in disease conditions [36]. The Fig. 3 and Table 2 show the levels of hepatic and renal function markers respectively in all the experimental groups of rats. Ethanol intoxication, significantly (p<0.05) increased the activities of ALT, AST, ALP, and GGT as well serum Cr, urea and UA when compared with control rats. Whereas ethanol-fed rats along with NMJ and GMJ samples significantly reversed (p<0.05) the ethanol-induced increase in serum Cr, urea and UA levels as well the activities of ALT, AST, ALP, and GGT when compared with ethanol group. In addition, NMJ and GMJ treatments also brought back the activities of ALT, AST, ALP, and GGT as well as serum Cr, urea, and UA to near normal levels.
Serum levels of AST, ALT, and ALP are the most frequently utilized indicators of hepatocellular injury. An increased serum level of GGT served as an indicator of hepatobiliary disease and was also considered as a marker of oxidative stress because it catalyzes the first step in the degradation of extracellular glutathione [37]. In the present study a significant (p<0.05) increase in serum liver function enzymes (AST, ALT, ALP, and GGT) were observed in ethanol-treated rats compared to NC rats. Similar to our study significant rise in serum activities of ALT, AST, GGT, and ALP were reported in ethanol treated rats [38]. The administration of polyphenols present in pomposa fruit juice at levels 400, 800, and 1200 ppm did not cause any significant changes in the activities of serum AST, ALT, and ALP enzymes and were similar to the control group [39]. Similarly, the activity of the enzymes ALT and AST were not affected by the consumption of tropical fruit juices [40]. Our results also agree with the above observations.

It is also well known that serum Cr, urea, UA levels are consistent biochemical indices for renal function and their increases indicate the kidney function impairment such as acute glomerulonephritis [41]. In this study, ethanol intoxication caused significant (p<0.05) elevation of serum urea, Cr and UA levels (Table 2). Similar to this study increased serum urea, UA, and Cr levels were reported in ethanol treated rats [42]. 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 NMJ and GMJ treatment further confirmed the protective effect of these natural products against ethanol-induced renal dysfunction. Similarly, no significant changes in the levels of urea and UA were reported in rats administrated with different levels of phenolic compounds of pomposia fruit juice [39].

### Effect of ethanol and mango juice on the LPO in liver and kidney tissues

The level of LPO is a measure of membrane damage and alterations in structure and function of cellular membranes. The level of TBARS is an indirect measure of LPO [43]. In this study, there was a significant (p<0.05) increase in both liver and kidney TBARS levels of ethanol treated rats and no significant differences in remaining other groups, compared to control rats. However, NMJ and GMJ supplements with ethanol significantly (p<0.05) decreased the MDA production when compared with ethanol group. The administration of NMJ and GMJ alone for intact rats significantly (p<0.05) reduced the LPO product (MDA) when compared with ethanol-fed rats (Fig. 4).

In our study, we observed a significant increase in LPO during ethanol consumption, as reported by earlier studies [44]. The ethanol intoxication increases LPO production in various tissues and is indicative of tissue oxidative stress. Nearly, 60-80% ingested ethanol is metabolized in the liver, and this makes it more vulnerable than other organs to ethanol-induced oxidative stress [45]. This concept is supported by the larger extent of increase in LPO production in the liver, as compared to other organs. Mango juice co-treatment was found to suppress the ethanol-induced increase in oxidative stress, implicating a protection against ethanol-induced toxicity. The administration of pomegranate juice to rats induced a significant reduction in MDA of liver and kidney (37% and 33%, respectively), indicating that pomegranate has antioxidant properties [46]. Phenolic compounds derived from pomegranate, have been used as antioxidant agents to prevent various LPO induced damages in different organs [47]. Similarly, mango juice containing various bioactive compounds such as carotenoids [48] and polyphenolic compounds [14] contributed to antioxidant property and prevented various LPO induced damages in liver and kidney.

**Effect of ethanol and mango juice on non-enzymatic antioxidants: Vitamin C and GSH levels in liver and kidney**

The hepatic and renal levels of vitamin C and GSH were significantly (p<0.05) decreased in rats treated with ethanol when compared with NC. However, NMJ and GMJ supplements significantly (p<0.05) increased vitamin C and GSH levels in both liver and kidney, when compared with ethanol group. The administration of NMJ and GMJ alone for intact rats significantly (p<0.05) increased the hepatic and renal levels of vitamin C and GSH when compared with ethanol-fed rats (Fig. 5).

Vitamin C is a major hydrophilic antioxidant in both plasma and the cytosol of many cells. It contributes to the neutralization of many water-soluble oxidants and acts synergistically with vitamin E to terminate radical-induced lipid oxidation [49]. Vitamin C has also been reported to contribute up to 24% of the total peroxyl radical-trapping antioxidant activity [50]. In the present study vitamin C concentration was found to be significantly (p<0.05) decreased in the liver and kidney of ethanol treated rats (Fig. 5). Similar to the present study, vitamin C concentration was found to be significantly decreased in the kidney of ethanol treated rats [51]. This decrease in vitamin C level in tissues (liver and kidney) may be due to increased utilization for scavenging the free radicals and decreased GSH level, which is required for recycling of vitamin C.

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**Table 2: Effect of ethanol and mango juice on serum urea, creatinine and uric acid levels in normal and experimental animals**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Uric acid (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>34.7±6.1</td>
<td>0.54±0.05</td>
<td>1.38±0.21</td>
</tr>
<tr>
<td>EtOH</td>
<td>52.4±8.5</td>
<td>1.02±0.09</td>
<td>1.94±0.46</td>
</tr>
<tr>
<td>NMJ</td>
<td>32.8±4.7</td>
<td>0.52±0.03</td>
<td>1.31±0.24</td>
</tr>
<tr>
<td>GMJ</td>
<td>31.2±5.4</td>
<td>0.51±0.04</td>
<td>1.35±0.19</td>
</tr>
<tr>
<td>EtOH+NMJ</td>
<td>38.7±6.3</td>
<td>0.88±0.07</td>
<td>1.62±0.28</td>
</tr>
<tr>
<td>EtOH+GMJ</td>
<td>34.3±7.2</td>
<td>0.79±0.06</td>
<td>1.53±0.37</td>
</tr>
</tbody>
</table>

Values are given as mean±SD of six rats in each group. Values not sharing a common superscript letter in a column differ significantly at p<0.05 (DMRT). SD: Standard deviation, NC: Normal control, NMJ: Non-irradiated mango juice, GMJ: γ-irradiated mango juice, DMRT: Duncan's multiple range test

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**Fig. 3: Effect of ethanol and mango juice on serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and gamma-glutamyl transeptidase of normal and experimental animals**

**Fig. 4: Effect of ethanol and mango juice on the lipid peroxidation in liver and kidney tissues of normal and experimental rats**
GSH, a non-enzymatic antioxidant is known to accord protection against reactive O\textsubscript{2} species by effectively scavenging free radicals and other ROS directly and indirectly through enzymatic reactions. The levels of GSH were significantly decreased in ethanol-treated rats, as well as in earlier published reports, which shows that the GSH concentration decreases during ethanol ingestion [44,52]. The GSH level was significantly reduced in ethanol-treated animals; the reverse condition was observed in the co-treatment with NMJ and GMJ. The effect of mango juice on GSH level is due to its polyphenols, where it is known to be able to modulate the transcription and expression of proteins related to the endogenous antioxidant defense by interacting with antioxidant response elements in gene promoter regions of genes encoding proteins related to oxidative injury management [53].

The observed decrease in the levels of liver and kidney vitamin C and GSH in ethanol-fed rats could be the results of increased use of these antioxidants in scavenging the ethanol-overproduced free radicals. In the present study, co-treatment of ethanol-intoxicated rats with NMJ and GMJ increased the levels of vitamin C and GSH. This may be due to the effect of mango juice antioxidant compounds on the ROS, produced during ethanol metabolism. Thus, mango juice may exert a beneficial effect in neutralizing the toxic free radicals in liver and kidney.

**Effect of ethanol and mango juice on the antioxidant enzymes of liver and kidney (SOD, CAT, GPx, GR, and GST)**

The activity of all the liver and kidney antioxidant enzymes, such as SOD, CAT, GPx, GR, and GST significantly (p<0.05) decreased in ethanol-treated rats when compared to control. However, NMJ and GMJ co-treatment significantly (p<0.05) increased SOD, CAT, GPx, GR, and GST activities in both liver and kidney when compared with the ethanol group. The administration of NMJ and GMJ alone for intact rats significantly (p<0.05) increased all the liver and kidney antioxidant enzymes activities compared to control rats (Figs. 6-10).

Liver and kidney are the two important organs which play an important role in physiological aspects; any damage in these tissues can lead to
alterations in metabolic activities including cellular antioxidant status. The biological effects of ROS are controlled in vivo by a wide spectrum of enzymatic defense mechanisms. SOD, one of the first antioxidant enzymes in the line of defense against the deleterious effects of oxygen radicals in the cells, scavenges ROS by catalyzing the dismutation of superoxide to hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) [44,52]. In the present study, the SOD activity was significantly reduced in liver and kidney tissues of ethanol-intoxicated rats. The SOD activity returned to normal levels with co-treatment of NMJ and GMJ. CAT acts as a preventive antioxidant and plays an important role in the protection against the deleterious effects of lipid peroxidase. Reports have shown that there is a significant decrease in the activities of CAT in alcoholic subjects [44]. Our results also agree with the above observations. A decreased activity of CAT was due to exhaustion of the enzyme as a result of oxidative stress caused by ethanol. Presumably, a decrease in CAT activity could be attributed to cross-linking and inactivation of the enzyme protein by lipid peroxides [44]. The CAT activity was restored to normal with co-treatment of NMJ and GMJ, which shows the antioxidant property of the mango juice against oxygen free radicals. Thus, the analysis of antioxidant status in our study indicates that enzymatic antioxidants were decreased due to ethanol-induced toxicity.

GPx plays a pivotal role in \( \text{H}_2\text{O}_2 \) catabolism and the detoxification of endogenous metabolic peroxides and hydroperoxides, which catalyses GSH level. Decreased GPx activity after chronic exposure to ethanol was observed in our study as observed by others [44,52]. The decreased level of GPx in the liver and kidney of rats that ingested ethanol could be due to either free radical-dependent inactivation of enzyme or depletion of its co-substrates, that is GSH and NADPH [44]. GR also known as glutathione disulfide reductase (GSR) is an enzyme that in humans are encoded by the GSR gene. It is also one of the GSH-related enzymes which play detoxifying and antioxidant roles in metabolizing xenobiotics through the conjugation with glutathione or reduction of free radicals. GR is concerned with the maintenance of cellular level of GSH (especially in the reduced state) by effecting rapid reduction of oxidized glutathione (GSSG) to reduced form (GSH) by the oxidation of NADPH to NADP+. Deficiency of this enzyme will affect the redox status of GSH in biological system and inability to protect tissues from oxidative damage. The decrease in liver and kidney GR activity in the rats treated with ethanol might result from utilization of this enzyme for GSSG reduction or from NADPH deficiency [44]. The reversal of the GPx and GR activities is presumably due to the antioxidant bioactive compounds and other detoxifying metabolic products of mango juice. GST previously known as ligandin comprises a family of eukaryotic and prokaryotic Phase II metabolic isozymes best through the action of conjugation with GSH, metabolize xenobiotics such as carcinogens and pollutants as well as by-products of oxidative stress for the purpose of detoxification. The activity of GST is dependent upon a steady supply of GSH from the synthetic enzymes γ-glutamylcysteine synthetase and glutathione synthetase, as well as the action of specific transporters to remove conjugates of GSH from the cell. The primary role of GST is to detoxify xenobiotics by catalyzing the nucleophilic attack by GSH on electrophilic carbon, sulfur, or nitrogen atoms of said nonpolar xenobiotic substrates, thereby preventing their interaction with crucial cellular proteins and nucleic acids [54]. The GST level was significantly reduced in ethanol treated rats, and an upward reversal of the condition was observed at co-treatment with NMJ and GMJ. This may be attributed to a direct action of the mango juice on the hepatic and renal GST activation, the mechanism of which is not known.

**Histopathological observations in liver tissue**

Liver is considered to be consisting of large number of hexagonal lobules. Each lobule consists of a central vein, from which cords or rows of liver cells radiate like spokes of a wheel. Each lobule is delineated by connective tissue. The photomicrograph of the liver of an NC rat showing the normal hepatic architecture considered to consist of normal central vein, prominent nucleus and normal hepatocytes (Fig. 11). The liver of rats treated with ethanol showed degenerative liver with severe congestion of central vein, hemorrhages in the sinusoidal spaces and granular appearance of the hepatocytes (degenerative change) with cloudy swelling (hazy nucleus) (Fig. 11). Similar changes in the liver in rats induced by ethanol have been reported earlier [55]. The above pathological changes were reversed on co-treatment with NMJ and GMJ. The ethanol treated rats along with NMJ and GMJ supplementation allowed the hepatic cells, hepatocytes, and sinusoids to regain this normal structure. No significant alterations (normal architecture similar to control rats) were also observed in liver photomicrographs of NMJ and GMJ alone treated rats (Fig. 11). Similar to this study, liver and kidney tissues of rat administered with various levels of pomposa juice had the same character as that of control rats [39]. Thus, mango juice minimized the production of free radicals in ethanol-treated rats, and hence the liver tissue morphology was restored from the free radical damage in these groups.

**Histopathological observations in kidney tissue**

The photomicrograph of the kidney of an NC rat showing normal architecture of kidney considered to consist of normal glomeruli and normal tubular epithelial cells (Fig. 12). In ethanol treated rats, severe tubular degeneration, degeneration of glomeruli, focal necrosis of tubules, cystic dilatation of tubules, congestion of blood vessels, and fatty infiltration were observed in the kidney tissue (Fig. 12). Similar changes in the kidney, in rats induced by ethanol, have been reported earlier [44,52]. The above pathological changes were reduced in NMJ and GMJ co-treated rats. In kidney tissue, mango juice diminished the production of free radicals after ethanol treatment, and the structural functionality of the tissue was re-established from the free radical toxicity.
damage in these groups. No significant alterations (normal architecture similar to control rats) were also observed in kidney photomicrographs of NMJ and GMJ alone treated rats (Fig. 12). Similar to this study; no visible lesions were observed in the kidney of rats treated with Opuntia ficus indica f. inermis prickly pear's juice alone [51].

Histopathological studies of kidney revealed severe tubular degeneration, degeneration of glomeruli, focal necrosis of tubule and fatty infiltration in ethanol treated rats, which might be associated with increased diuresis; the collecting tubules showed dilatation probably under the pressure of increased urine flow and renal hypertrophy in ethanol treated rats. The above pathological changes were reduced in NMJ and GMJ co-treated rats. The glomeruli appear to be restored; the tubules also appear to be regenerated and less fatty infiltration was observed in the ethanolic rats co-treated with mango juice, which may be due to a protective effect of mango juice. Thus, the histopathological observations support, with visual histological evidence, that the mango juice produced a significant increment of antioxidant enzymes and protected the hepatic and renal tissues in ethanol-treated rats.

CONCLUSION
The present report is the first study examining the effect of mango juice on ethanol-inducing liver and kidney injury. This study confirms that ethanol-induced hepato-renal toxicity in terms of decreased antioxidant status, increased LPO and damage to hepatic and renal cells. However, all these adverse effects were reversed by mango juice supplementation. The bioactive components of mango juice could act synergistically in preventing ethanol toxicity, by scavenging free radicals and ROS, by stabilizing hepatic and renal cells membrane integrity and restoring antioxidant enzymes levels. Thus, this data suggest that mango juice can be used as a protective agent and as an antioxidant supplement to protect the liver and kidney from ethanol-induced oxidative damage.

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