academicJournals

Vol. 9(10), pp. 335-342, 10 March, 2015
DOI: 10.5897/JMPR2015.5757
Article Number: D8DB79E51702
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Assessment of antioxidant capacity of ethanolic extract of *Portulaca oleracea* leaves *in vitro* and *in vivo*

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Received 4 February, 2015; Accepted 3 March, 2015

The purpose of the present study was to prepare ethanol extract of *Portulaca oleracea* L. leaves (PLE) that is rich in phenolic compounds and investigate there *in vitro* and *in vivo* antioxidative activities using ethanol to induce oxidative stress in the liver. This study includes two phases. The first phase includes assessment of antioxidant capacity *in vitro* using 1,1- Diphenyl-2-picrylhydrazyl (DPPH) assay and 2,2-azinobis-(3-ethyl-benzothiazo-line-6-sulfonic acid) (ABTS) assay. In the second phase (assessment of antioxidant capacity *in vivo*), the levels of antioxidant parameters in the alcoholic liver disease rats were studied and the protective effect of ethanolic extract of *P. oleracea* L. in the alcoholic liver disease rats was examined to further understand their mechanisms. *In vitro* studies with DPPH and ABTS showed that PLE possesses antioxidant activity. *In vivo* administration of ethanol (7.9 g/kg body weight/day) for 45 days with PLE (100 mg/kg body weight/day) significantly decreased the activities of liver markers enzyme serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and gamma glutamyl transferase in serum towards near normal level. In addition, PLE also significantly reduced the levels of lipid peroxidation and in addition, significantly restored the enzymatic and non-enzymatic antioxidants level in the liver of alcohol administration rats. Our study suggests that ethanol extract of *P. oleracea* leaves play a beneficial role in the treatment of alcohol induced tissue damage, which could be one of its therapeutic values.

Key words: Alcoholic liver disease, *Portulaca oleracea* L., oxidative stress, ethanolic extract, antioxidants, rats.

INTRODUCTION

Oxidative stress is an important factor in the genesis of many pathologies, from cancer to cardiovascular and degenerative diseases (Gul et al., 2014; Zia-Ul-Haq et al., 2014 a,b; Galli et al., 1999; Parthasarathy et al., 2001; Cooke et al., 2003, Soltani et al., 2009). Oxidative stress can cause damage to lipids, proteins, and nucleic acids, resulting in changes in signal transduction pathways, gene expression, cell mutagenesis, and cell death (Baratta, 2000). Alcoholic liver disease is a major cause of morbidity and mortality worldwide. Patients with cirrhosis caused by alcohol are at risk for developing complications associated with a failing liver. The long-term management of alcoholic liver disease stresses the following: Abstinence of alcohol (Grade 1A), with referral to an alcoholic rehabilitation program; Adequate nutritio-nal support (Grade 1B), emphasizing multiple feedings

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and a referral to a nutritionist; Routine screening in alcoholic cirrhosis to prevent complications; Timely referral to a liver transplant program for those with decompensated cirrhosis; Avoid pharmacologic therapies, as these medications have shown no benefit.

Antioxidants play an important role in the human body by reducing oxidative reactions. Especially, endogenous antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and non-enzymatic antioxidants such as vitamin C, α-tocopherol and selenium protect internal organs and tissues from oxidative damage by various toxic reactive oxygen and nitrogen species (Ahn et al., 2004). In order to protect the body against the consequences of oxidative stress, an efficacious approach consists in improving the antioxidant nutrition. In this regard, scientific studies have shown that antioxidants from natural sources have a higher bioavailability and therefore higher protective efficacy than synthetic antioxidants (Gey, 1998). Focusing our attention on natural and bio-available sources of antioxidants, we undertook to investigate the antioxidant properties of *P. oleracea* L. leaves extract. *P. oleracea* has been shown to display radical scavenging activity (Lim and Quah, 2007; Oliveira et al., 2009; Siriamornpun and Suttajit, 2010).

P. oleracea L., belonging to the Portulacaceae family, is a warm-climate, herbaceous succulent annual plant with a cosmopolitan distribution. It is eaten extensively as a potherb and added in soups and salads around the Mediterranean and tropical Asian countries and has been used as a folk medicine in many countries. Diverse compounds have been isolated from *P. oleracea*, such as flavonoids, alkaloids, polysaccharides, fatty acids, terpenoids, sterols, proteins vitamins and minerals. *P. oleracea* possesses a wide spectrum of pharmacological properties such as neuroprotective, antimicrobial, antidiabetic, antioxidant, anti-inflammatory, antiulcerogenic, and anticancer activities (Zhou et al., 2015).

P. oleracea is listed in the World Health Organization as one of the most used medicinal plants and it has been given the term 'Global Panacea' (Chu et al., 2002; Katalinic et al., 2006). Thus, the purpose of the present study was to prepare ethanol extract of *P. oleracea* L. leaves that is rich in phenolic compounds and investigate their *in vitro* and *in vivo* antioxidative activities using ethanol to induce oxidative stress in the liver.

MATERIALS AND METHODS

Chemicals and reagents

Ethanol, ascorbic acid and potassium persulfate were obtained from Merck (Germany). 1,1- Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma (St. Louis, MO). 2,2-azinobis-(3-ethyl-benzothiazo-line-6-sulfonic acid) (ABTS) was obtained from Fluka (Fluka Chemie Gmbh, Stenheim, Germany). All other reagents were of analytical grade.

Plant

Fresh *P. oleracea* leaves were collected from the farm of Faculty Agricultural, Zagazig University, Egypt. The plant material was stored at room temperature in a dry place prior to use.

Animals

Healthy male white albino rats (*Rattus norvegicus*), Wistar strain (160 ± 10 g, body weight) were obtained from Organization of Biological Products and Vaccine (Helwan farm, Cairo, Egypt) and housed in plastic cages in groups of 5 animals/cage. The experimental animals were allowed to acclimatize under the laboratory conditions (temperature of 25 ± 5°C; relative humidity 50 to 70% and normal light/dark cycle) for 2 weeks at least prior the experiment. They were provided with balanced pelleted diet (23% protein) and tap water *ad libitum* throughout the adaptation and experimental period.

Sample preparation and extraction

Air dried leaves of *P. oleracea* L. were dried in a vacuum oven at 40°C and ground to a fine powder in a mill. Ground material (10 g) was extracted with ethanol 80% (100 ml) using magnetic stirrer at room temperature for 72 h followed by filtration through Whatman No.1 filter paper and concentrated to dryness with a rotary evaporator (BÜCH-water bath-B-480, Switerland) and lyophlized by Freeze-Dryer (Thermo-Electron Corporation-Heto power dry LL300 Freeze Dryer, Czech Republic). The dried extract (PLE) after evaporation and lyophlization was stored at -20°C until further use.

Assessment of antioxidant capacity *in vitro*

DPPH radical-scavenging activity assay

The electron donation ability of the obtained extract was measured by bleaching of the purple colored solution of DPPH according to the method of Hanato et al. (1988) with slight modification. One milliliter of *P. oleracea* leaves ethanolic extract (PLE) at different concentration (0, 50, 100, 150 and 200 µg ml^{-1}) was mixed with 4 ml of 0.15 mM DPPH (in 80% ethanol). The mixture was then shaken vigorously using a mixer. The reaction mixture was incubated for 30 min in the darkness at room temperature. The absorbance of the resulting solution was measured at 517 nm using JENWAY 6405 UV/visible spectrophotometer (UK). Ethanol 80% and ascorbic acid was used as a control and standard sample, respectively.

Percentage of antioxidant activity of free radical DPPH was calculated as follow:

$$\text{Antioxidant activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

The IC_{50} value was defined as an effective concentration of extract that is required to scavenging 50% of radical activity. All experiments were carried out in triplicate.

$ABTS^{\bullet+}$ radical-scavenging activity assay

For the $ABTS^{\bullet+}$ assay, the method of Re et al. (1999) was adopted. The stock solutions were 7 mmol l^{-1} $ABTS^{\bullet+}$ solution and 2.4 mmol l^{-1} potassium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 to 16 h at room temperature in the dark. One milliliter of the resulting $ABTS^{\bullet+}$ solution was diluted with 60 ml of methanol. $ABTS^{\bullet+}$ solution was freshly prepared for each

assay. Ten microliters of each extract (0, 50, 100, 150 and 200 µg ml^{-1}) was allowed to react with 5 ml of $ABTS^{\bullet+}$ solution for 7 min, then the absorbance at 734 nm was recorded. A control with no added extract was also analyzed. Scavenging activity was calculated as follows:

$ABTS^{\bullet+}$ radical-scavenging activity (%) = $[(Abs_{control} - Abs_{sample})/ Abs_{control}] \times 100$

where $Abs_{control}$ is the absorbance of $ABTS^{\bullet+}$ radical+methanol and Abs_{sample} is the absorbance of $ABTS^{\bullet+}$ radical + extract/synthetic antioxidant.

The IC_{50} value was defined as an effective concentration of extract that is required to scavenging 50% of radical activity. All experiments were carried out in triplicate.

Assessment of antioxidant capacity *in vivo*

In the present study, alcoholic liver disease rat's model is established. Furthermore, the levels of antioxidant parameters in the alcoholic liver disease rats were studied and the protective effect of ethanolic extract of *P. oleracea* L. leaves in the alcoholic liver disease rats was examined to further understand their mechanisms.

Experimental design

The animals were randomly divided into six groups of six rats in each group. The extract and alcohol was administered as aqueous solution using intragastric tube daily for 45 days.

Group 1: Control rats (normal rats) treated with 30% glucose (isocaloric to ethanol) and 0.1% carboxymethylcellulose (CMC);

Group 2: Control rats orally received PLE (100 mg/kg body weight) suspended in 0.1% CMC; Group 3: Normal rats orally received 20% ethanol (3.95 g/kg body weight twice a day that is 7.9 g/kg/day) (Rajakrishnan et al., 1997); Group 4: Normal rats orally received 20% ethanol with PLE (25 mg/kg body weight); Group 5: Normal rats orally received 20% ethanol with PLE (50 mg/kg body weight); Group 6: Normal rats orally received 20% ethanol with PLE (100 mg/kg body weight).

Preparation of serum, plasma and tissue homogenate

At the end of the experimental period, the animals were sacrificed by cervical decapitation. Blood was collected and centrifuged for serum separation. For plasma, blood was collected with anticoagulant and centrifuged (2000 *g* for 20 min) to separate plasma. The tissues were dissected out, weighed and washed using ice cold saline solution. Tissues were minced and homogenized (10% w/v) in Tris-HCl buffer (0.1M; pH 7.4) and centrifuged at 3000 *g* for 20 min at 4°C. The resulting supernatant was used for various biochemical assays.

Biomarkers of oxidative stress

The activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assayed spectrophotometrically according to the standard procedures using commercially available diagnostic kits. Gamma glutamyl transferase (GGT) activity was determined by the method of Rosalki and Rau (1972). Superoxide dismutase (SOD) content was measured using the technique of Kakkar et al. (1984). A single unite of enzyme was expressed as 50% inhibition of nitroblue tetrazolium (NBT) reduction/min/mg protein. The catalase (CAT) activity was measured calorimetrically at 620 nm and expressed as 1 mol of H_2O_2 consumed/min/mg protein as described by Sinha (1972). The glutathione peroxidase (GPx) activity was measured using the Ellman (1959).

Ascorbic acid (vitamin C) concentration was measured using Omaye et al. (1979) method. Vitamin E concentration was measured using Desai (1984) method. Reduced glutathione (GSH) activity was determined using Ellman (1959) method. Lipid hydroperoxides as evidenced by formation of TBARS and hydroperoxides (HP) were measured using Niehius and Samuelsson (1968) and Jiang et al. (1992) methods, respectively.

Histopathological investigation

The liver samples were fixed for 48 h in 10% formal-saline, and were dehydrated by passing successfully in different mixture of ethyl alcohol-water, cleaned in xylene and embedded in paraffin. Section of liver (5 to 6 µm thick) were prepared and then stained with hematoxylin and eosin dye (H&E), which was mounted in neutral distyrene-dibutyl phthalate–xylene (DPX) medium for microscopic observations.

Statistical analysis

The data for various biochemical parameters were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using statistics software package (SPSS for Windows, V. 13.0, Chicago, USA). P values <0.05 were considered as statistically significant.

RESULTS

Assessment of antioxidant capacity *in vitro*

Figure 1 shows the antioxidant activities of ethanolic *P. oleracea* leaves extract at different concentrations (50, 100, 150 and 200 µg ml^{-1}) against DPPH and ABTS radicals. The ethanolic extract of *P. oleracea* leaves exhibited a significant inhibition on DPPH and ABTS radicals in a dose dependent manner. The 50% inhibition (IC_{50}) of DPPH and ABTS radicals were shown in a concentration of 116.25 ± 2.74 and 89.73 ± 5.56 µg ml^{-1}, respectively. The IC_{50} values of standard ascorbic acid are 46.17 ± 1.74 and 29.72 ± 2.53 µg ml^{-1}.

Assessment of antioxidant capacity *in vivo*

Liver AST, ALT, ALP and GGT activities

The results of the changes in activities of the serum enzymes, AST, ALT, ALP and GGT are represented as shown in Table 1. The result shows that ethanol administration significantly ($P < 0.05$) increased the activities of AST, ALT, ALP and GGT (165.91 ± 12.43, 65.02 ± 5.47, 130.92 ± 9.28, and 5.85 ± 0.41 IU l^{-1}), respectively. Administration of PLE along with alcohol significantly ($P < 0.05$) reversed these functional markers towards near normal in a dose dependent manner. PLE at a dose of 100 mg/kg body weight was more effective (80.81 ± 6.08, 25.60 ± 1.99, 83.47 ± 7.38 and 2.18 ± 0.28 IU l^{-1}),

Table 1. Liver AST, ALT, ALP and GGT after daily administration for 45 days of ethanolic extract of *P. oleracea* L. leaves (PLE) at different concentration (25, 50 and 100 mg/kg body weight) in normal and alcoholic liver disease rats (20% ethanol) compared to control.

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)
Control	74.29 ± 5.44	23.89 ± 2.39	77.79 ± 4.71	2.14 ± 0.19
Control + PLE (100 mg/kg)	73.56 ± 4.68	20.71 ± 2.16	77.85 ± 5.41	1.83 ± 0.12
20% Ethanol	165.91 ± 12.43	65.02 ± 5.47	130.92 ± 9.28	5.85 ± 0.41
20% Ethanol + PLE (25 mg/kg)	153.07 ± 10.09	50.16 ± 5.23	115.92 ± 8.40	4.68 ± 0.49
20% Ethanol + PLE (50 mg/kg)	120.53 ± 11.26	40.60 ± 3.72	95.40 ± 5.52	3.63 ± 0.33
20% Ethanol + PLE (100 mg/kg)	80.81 ± 6.08	25.60 ± 1.99	83.47 ± 7.38	2.18 ± 0.28

Values are given as mean ± SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

Table 2. Levels of lipid peroxidation markers (TBARS and lipid hydroperoxides) in liver tissue after daily administration for 45 days of ethanolic extract of *P. oleracea* L. leaves (PLE) at 100 mg/kg body weight in normal and alcoholic liver disease rats (20% ethanol) compared to control.

Group	TBARS (mM/100 g tissue)	Lipid hydroperoxides (mM/100 g tissue)
Control	0.77 ± 0.05	91.92 ± 8.24
Control + PLE (100 mg/kg body weight)	0.78 ± 0.05	83.31 ± 5.27
20% Ethanol	2.02 ± 0.14	160.27 ± 11.26
20% Ethanol + PLE (100 mg/kg body weight)	0.84 ± 0.08	103.26 ± 9.01

Values are given as mean ± SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

Table 3. Levels of non-enzymatic antioxidants (Vitamin C, Vitamin E and GSH) in liver tissue after daily administration for 45 days of ethanolic extract of *P. oleracea* L. leaves (PLE) at 100 mg/kg body weight in normal and alcoholic liver disease rats (20% ethanol) compared to control.

Group	Vitamin C (µmol/mg tissue)	Vitamin E (µmol/mg tissue)	GSH (µmol/mg tissue)
Control	1.91 ± 0.11	2.21 ± 0.11	50.55 ± 3.39
Control + PLE (100mg /kg bw)	1.71 ± 0.11	1.59 ± 0.12	49.59 ± 3.62
20% Ethanol	0.91 ± 0.71	1.04 ± 0.06	27.95 ± 1.99
20% Ethanol + PLE (100 mg/kg bw)	1.35 ± 0.10	1.51 ± 0.10	40.16 ± 2.91

Values are given as mean ± SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at $P < 0.05$ (DMRT).

respectively when compared with two other doses (25 and 50 mg/kg body weight). Hence, 100 mg/kg body weight was used for further studies.

Table 2 shows that the level of peroxidation products such as TBARS and lipids hydroperoxide in liver tissue of control and experimental rats. A significant elevation in the levels of lipid peroxidation markers [TBARS (2.02 ± 0.14 mM/100 g tissue) and lipids hydroperoxide (160.27 ± 11.26 mM/100 g tissue)] were observed in ethanol fed group when compared with control rats (0.77 ± 0.05 and 91.92 ± 8.24 mM/100 g tissue), respectively. Administration of PLE (100 mg/kg body weight) along with ethanol caused a significant decrease in the level of TBARS (0.84 ± 0.08 mM/100 g tissue) and lipid hydroperoxide (103.26 ± 9.01 mM/100 g tissue) in liver when compared with alcohol administrated rats.

Table 3 represents the levels of non-enzymatic antioxidants (vitamin E, vitamin C and GSH) status in tissues. The levels of vitamin C, vitamin E and GSH were significantly ($P < 0.05$) reduced in alcohol treated rats (0.91 ± 0.71, 1.04 ± 0.06 and 27.95 ± 1.99 µmol/mg tissue), respectively when compared with control rats (1.91 ± 0.11, 2.21 ± 0.11 and 50.55 ± 3.39 µmol/mg tissue), respectively. Administration of PLE (100 mg/kg

Table 4. The activities of antioxidant enzymes (SOD, CAT, GPx and GST) in liver tissue after daily administration for 45 days of ethanolic extract of *P. oleracea* L. leaves (PLE) at 100 mg/kg body weight in normal and alcoholic liver disease rats (20% ethanol) compared to control.

Group	SOD (U^A/mg protein)	CAT (U^A/mg protein)	GPx (U^A/mg protein)	GST (U^A/mg protein)
Control	9.57 ± 0.73	78.88 ± 6.56	9.95 ± 0.85	6.81 ± 0.44
Control + PLE(100 mg/kg bw)	9.89 ± 0.87	84.65 ± 6.59	10.07 ± 0.80	6.78 ± 0.46
20% Ethanol	4.77 ± 0.39	56.41 ± 5.47	5.82 ± 0.44	4.26 ± 0.26
20% Ethanol + PLE (100 mg/kg bw)	9.02 ± 0.73	74.65 ± 7.40	9.46 ± 0.55	5.75 ± 0.36

Values are given as mean ± SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at P < 0.05 (DMRT). [A]Units of enzyme activities are expresses as: SOD – One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1 min. CAT – µmol of hydrogen peroxide consumed/min. GPx – µg of glutathione consumed/min. GST – µmol of CDNB – GSH conjugate formed/ min.

body weight) to alcohol treated rats significant (P < 0.05) restored the levels of non-enzymatic antioxidants in tissues.

The activities of antioxidant enzymes, namely, superoxide dismutase (SOD), catalase (CAT), glutathione peroxide (GPx) and glutathione-S-transferase (GST) in liver were given as shown in Table 4. A significant (P < 0.05) decrease in the activities of enzymatic antioxidants were observed in alcohol treated rats (4.77 ± 0.39, 56.41 ± 5.47, 5.82 ± 0.44 and 4.26 ± 0.26 U/mg protein), respectively when compared with control rats (9.57 ± 0.73, 78.88 ± 6.56, 9.95 ± 0.85 and 6.81 ± 0.44 U/mg protein), respectively. Administration of PLE (100 mg/kg body weight) to alcohol treated rats significantly (P < 0.05) increased the activities of enzymatic antioxidants (9.02 ± 0.73, 74.65 ± 7.40, 9.46 ± 0.55 and 5.75 ± 0.36 U/mg protein), respectively when compared with alcohol treated rats.

Histological slices of rats liver were examined at the end of the experimental period. Results are as shown in Figure 2. The liver samples of alcohol-administered rats showed the focal hepatocytes damage and degeneration (Figure 2C). The administration of alcohol along with PLE (100 mg/kg body weight) showed near normal appearance hepatocytes (Figure 2D). The control (Figure 2A) and PLE (Figure 2B) alone treated rats showed normal appearance of liver.

DISCUSSION

The antioxidant activity of the plant extracts and standard were assessed on the basis of the radical scavenging effect on the stable DPPH and ABTS free radicals. A wide variety of *in vitro* methods to assessed radical scavenging ability of certain agents from natural and synthetic source. DPPH free radical has been used to assess the ability of phenolic compounds to transfer labile hydrogen atoms to radicals (Goupy et al., 2003). Our results showed the capacity of PLE had effective DPPH and ABTS radical scavenging activity in a concentration dependent manner. There is a significant decrease (P < 0.05) in the concentration of DPPH and ABTS due to the scavenging capacity of PLE. Several polyphenol including flavonoids are found in *P. oleracea* leaves (Brand-Williams et al., 1995). Generally, the chemical structure of flavan-3-ol family has good antioxidants response towards DPPH and ABTS radical. The hydrogen donating substituents (hydroxyl groups), attached to the aromatic ring structures of flavonoids, which enable the flavonoids to undergo a redox reaction that helps them to scavenge the free radicals (Brand-Williams et al., 1995).

AST and ALT are the reliable makers for liver function. It is established that AST can be found in the liver, cardiac muscle, skeletal muscle, kidney, brain, pancreas, lungs, leukocytes and erythrocytes whereas ALT presence in liver (Rej, 1997). The increased levels of serum enzyme such as AST and ALT indicate the increased permeability and damage and/or necrosis of hepatocytes (Goldberg and Watts, 1965). The membrane bound enzymes like ALP and GGT are released unequally into bloodstream depending on the pathological phenomenon (Sillanaukee, 1996). In our study, we have found that chronic ethanol consumption caused a significant increased in the activities of AST, ALT, ALP and GGT, which could be a severe damage to tissue membrane. The decreased activities of these enzymes on PLE administrated rats indicate the hepatoprotective effect of *P. oleracea* leaves extract.

Administration of PLE significantly decreased the level of lipid peroxidation when compared with alcohol treated rats, which may be due to the scavenging of free radicals generated by ethanol. It is known that the flavonoids found in *P. oleracea* leaves had inhibitory effect in lipid peroxidation by its free radical scavenging nature (Kravchenko et al., 2003). The flavonoids found in grape leaves such as anthocyanins, catechin and non-flavonoids (stilbene) has been reported to possess the capability to prevent the Fe^{2+}, Cu^{2+} induced lipid peroxidation (Fauconneau et al., 1997). Since the transition metals play a central role in lipid peroxidation process,

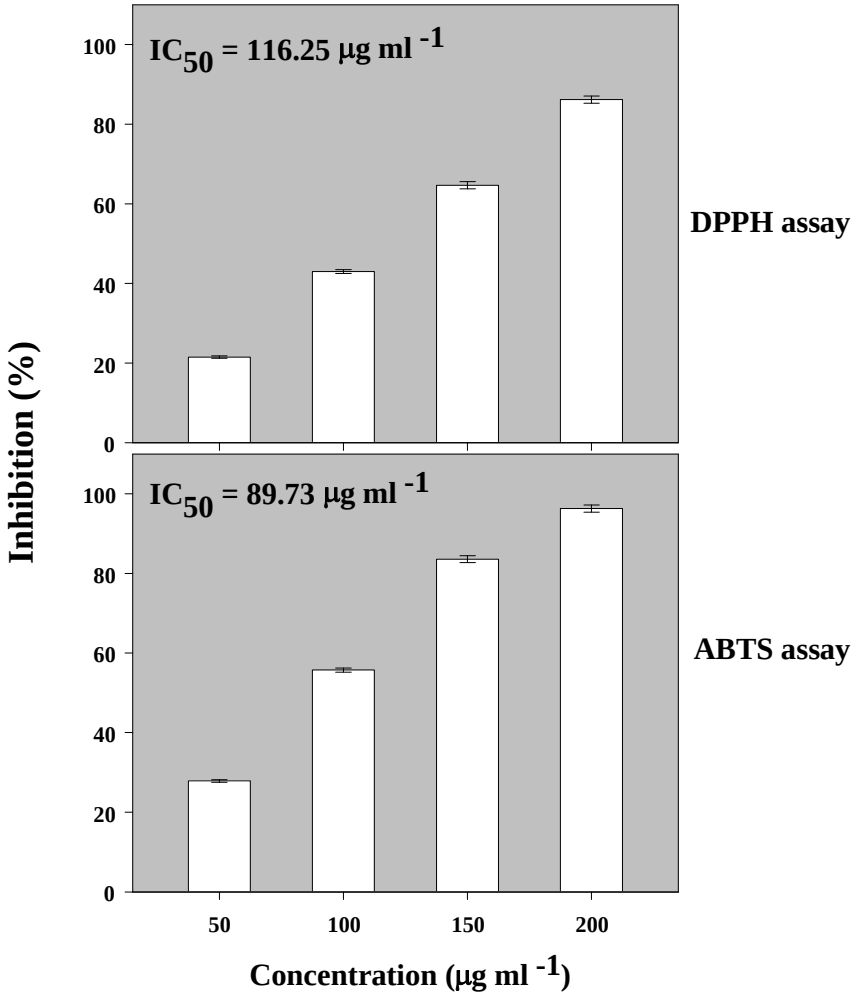

Figure 1. Scavening activity of ethanolic *P. oleracea* L. leaves extract at different concentrations (50, 100, 150 and 200 µg ml^{-1}) against DPPH and ABTS radicals.

our results obliviously indicate that purslane leaves extract has radical scavenging activity and inhibit the lipid peroxidation damage in alcohol toxicity.

Non-enzymatic antioxidants such as GSH, vitamin C and vitamin E are closely interlinked to each other and play an excellent role in protecting the cell from lipid peroxidation. The depleted level of GSH in alcohol toxicity may be due to scavenging of toxic radicals and inhibition of the synthesis and increased rates of turnover (Lieber, 1997). In addition to GSH, we have also observed a decrease in the levels of antioxidants such as vitamin C and E in tissue of alcohol treated rats. Supplementation of PLE to alcohol treated rats restored the non-enzymatic antioxidants levels in liver. The biologically active antioxidants found in *P. oleracea* leaves sparing the antioxidant activity and reduced the consumption of endogenous antioxidants, which could be responsible for the reduction of oxidative stress during ethanol toxicity. The active constituents such as flavanols, flavanol oligomers and proanthrocyanidins were found in *P. oleracea* leaves (Parry et al., 1993) and it has been reported as powerful antioxidants (Lim and Quah, 2007). This could be responsible for the reversal of antioxidants levels in tissues of alcohol fed rats treated with PLE.

Free radical scavenging enzymes such as SOD, CAT, GPx and GST are the first line of defense against oxidative injury. The inhibition of antioxidant system may cause the accumulation of H_2O_2 or products of its decomposition (Halliwell, 1994). SOD catalyzes the conversion of superoxide anion into H_2O_2. The primary role of catalase is to scavenge H_2O_2 that has been generated by free radical or by SOD in removal of superoxide anions.

The GSTs are a multigene family of isozymes that catalyze the conjugation of GSH to a variety of electrophilic compounds, and thereby exert a critical role in cellular protection against ROS (Hayes and Pulford, 1995). The

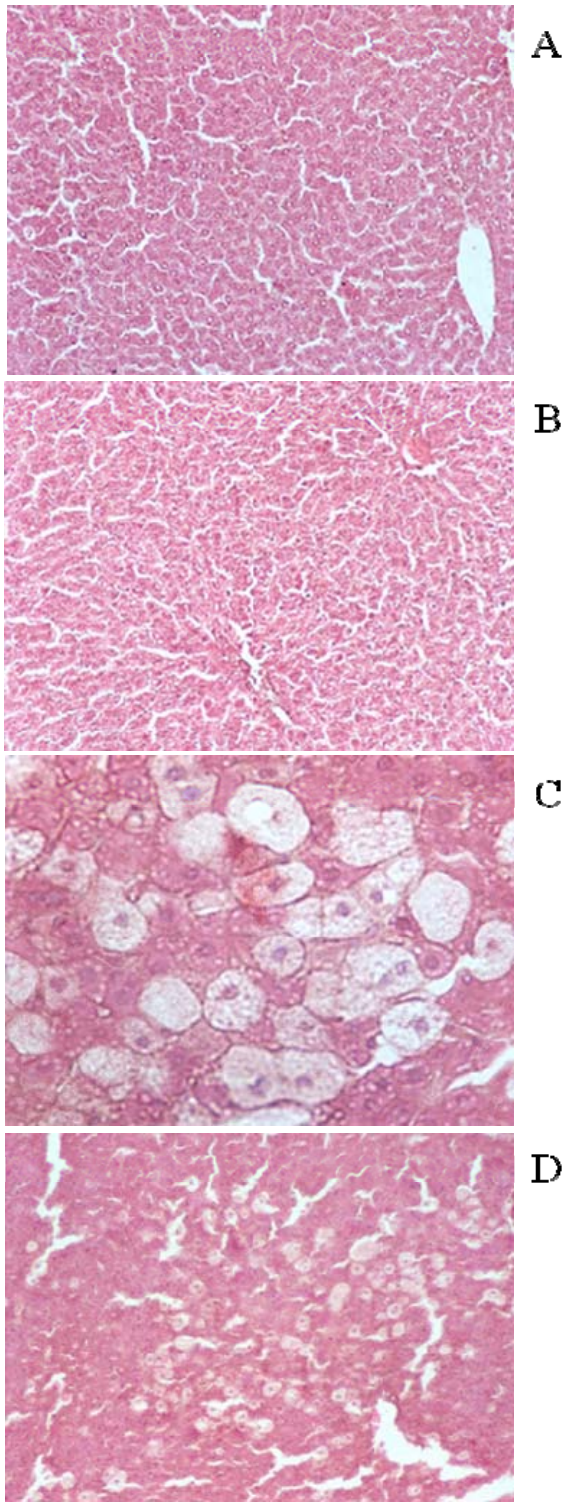

Figure 2. Histo-pathological examination of H&E stained sections of liver of male Albino rats after daily administration for 45 days of ethanolic extract of *P. oleracea* L. leaves (PLE) at 100 mg/kg body weight in normal and alcoholic liver disease rats (20% ethanol) compared to control. (A) control rat liver, (B) Control + PLE (100mg /kg body weight) rat liver, (C) alcohol treated rat liver and (D) 20% Ethanol + PLE (100 mg/kg body weight) rat liver.

detoxification of 4-hydroxynonenal, a toxic aldehyde generated from ethanol metabolism is compromised when GST activity is reduced. Thus, ethanol or its metabolic products might specifically target GST isoenzymes and the reduction in enzyme activity or expression may contribute to ethanol hepatotoxicity (Alin et al., 1985). The ethanol oxidation by CYP2E1 produces 1-hydroxy ethyl radicals, which have been shown to inactivate several proteins including antioxidant enzyme system (Epstein, 1996). In consistent with these reports, our results also showed that decreased activities of SOD, CAT, GPx and GST in tissues on the chronic alcohol treatment in rats. Administration of PLE restored the activities of enzy-matic antioxidant in liver and kidney. Polyphenolic compounds are present in *P. oleracea* leaves, which are powerful antioxidant properties, that are free radical scavenging activity (Lim and Quah, 2007).

The microscopic changes in the liver of alcohol treated rats predominant in the centrilobular region. Hepatic damage observed in the present study may be partially attributed to cytochrome P_{450} (Pieffer et al., 1979). Administration of PLE to alcohol treated rats reduced the liver cell damage and improved the histomorphology of the liver near to normal.

The results of functional tests together with histological observations suggest that alcohol leads to serious changes in histology of liver. The increased formation of lipid peroxides and associated reactive oxygen species leads to damage in membrane integrity and other pathological changes in liver. The efficacy of any protective drug is essentially dependent on its capacity of either reducing the harmful effects or in maintaining the normal physiology of cells and tissues, which have been attributed by toxins. The membrane protective properties and antioxidant nature of PLE might be helpful to alleviate the pathological changes caused by alcohol in liver and kidney.

Conclusion

Our data indicate that PLE (100 mg/kg body weight) has a protective action against alcohol-induced toxicity as evidenced by the lowered tissue lipid peroxidation and elevated levels of the enzymatic and non-enzymatic antioxidants in liver. Hence, our study suggests that PLE play a beneficial role in the treatment of alcohol induced tissue damage, which could be one of its therapeutic values.

Conflict of interests

The authors declared that there is no conflict of interests.

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