Biomacromol. J. Vol. 2, No. 1, 78-85, June 2016.

Biomacromolecular Journal
www.bmmj.org

Modulation of Fourier Transform Infrared Spectra and Copper Levels by Purslane (*Portulaca Oleracea)* Against Liver Necrosis Induced by Copper Sulphate

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(Received 29 August 2016, Accepted 3 November 2016)

ABSTRACT

Copper (Cu) is an essential trace element involved in normal reproduction but its over-exposure may produce some detrimental effects. The aim of this study was to investigate the effects of purslane on copper sulphate poisoning on liver structures changes. Twenty eight wistar rats were randomly allocated to four treatment groups. Group I) Control, Group II) Copper sulphate (200 mg/kg bw were applied by gavage daily for 4 wk, Group III) Purslane (gavage 400 mg/kg bw daily for 4wk) and Group IV) Combined treatment of copper sulphate and purslane as described in Group II and III. Animals were sacrificed after four weeks and the liver were removed for atomic absorption and Fourier transform infrared spectroscopy (FTIR). A significant increase in the levels of copper and liver weight of copper-treated rats was observed which, however, got moderated significantly upon purslane treatment. Moreover, based on the FTIR study, we confirmed that purslane administration significantly protected the macromolecular changes including, the lipid, protein, nucleic acid and specially carbohydrate structural damage that occurred in liver. The present study suggested that purslane have promising strategy to protect tissue from oxidative stress.

Keywords: Purslane, FT-IR Spectra, Copper level, Liver necrosis, Copper sulphate

INTRODUCTION

The liver plays a major role in regulating various physic-chemical functions of the body, including synthesis, secretion and metabolism of xenobiotics. The liver, unique in its capacity for regeneration following injury, may give rise to malignancy states of advanced fibrosis or cirrhosis [1,2].

Free radicals contribute towards tissue injury through covalent binding and lipid peroxidation whereas the compounds that can scavenge free radicals potentially ameliorate the liver injury [3,4]. Natural products and their purified compounds have received much attention as an alternative solution to numerous health problems as antioxidant agents in recent years [5]. Their antioxidant potential inhibits the generation of free radicals and exhibits significance in providing protection against hepatic damage [6].

*Portulaca oleracea*is a member of the purslane, Family "*portulacaceae*, the genus *Portulaca* contains about 40 topical and warms climate species. It is characterized by its taller upright growth habit and larger leaves and seeds [7,8]. The whole plant is considered antiphlogistic (takes the heat out), bactericide, antidiabetic, anaphrodisiac (opposite to aphrodisiac), emollient, calmative, diuretic, a refreshing agent [9]. The use of this plant as a vegetable, spice and medicinal plant has been known since the times of the ancient Egyptians and was popular in England during the middle Ages [10]. In addition, purslane may have a protective effect against oxidative stress caused by vitamin A deficiency [11]. Also, purslane contains active molecules for the treatment of some parasitic infectious diseases such as leishmaniasis and trypanosomiasis [12].

FTIR spectroscopy has shown rapid developments to be

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used as an efficient diagnostic tool [13]. FTIR has the ability to measure complex molecular vibrational modes that contain valuable information on changes occurring in various biomolecules during pathogenesis. A previous study reports that protective role of borneol, a natural terpene on liver metabolism in anitricoxide deficient model of hypertension through interpretation of FTIR spectral information and results demonstrate that FTIR can successfully indicate the molecular changes that occur in liver during oxidative stress [14]. This present study intends to evaluate the preventive potential of purslane against copper sulphate induced hepatic oxidative stress by atomic absorption and fourier transform infrared spectroscopy.

MATERIALS AND METHODS

Plant Juice

The fresh purslane herb was collected from the Jiroft in August 2016. Aqueous extract of purslane was prepared by the maceration method. An aqueous juice of the purslane herbs prepared by mashing in a proportion of 1:5 (w/v) in a sealed glass container and it was set aside for about 24 h. The resulting crude extract was filtered and dried in a bath of warm water [15].

Animals

Male wistar rats, (180-220 g) were purchased from Razi Research Institute of Kerman, Iran and kept in the Central Animal House, Department of basic sciences, University of Jiroft. The rats were housed in groups of seven per cage and maintained under standard laboratory conditions (12 h light: 12 h dark and 24 ± 2 ºC) during the experimental period. During the study, the animals received water and pellet food (Javaneh Khorasan Co, Iran) *ad libitum*. All investigations were conducted in accordance with the Guiding Principles for the Care and use of Research Animals and were approved by the Animal Ethics Committee at the department of basic sciences, University of Jiroft, Iran.

Experimental Design

Twenty-eight animals were randomly and equally assigned into four treatment groups. Animals in Group I served as normal controls and was given water and diet *ad libitum*. Animals in Group II were given orally copper in the form of copper sulphatepentahydrate ($CuSO_4.5H_2O$) (by gavage) every day at 200 mg/kg body weight for four wks [16]. Group III animals were given every day purslane at 400 mg/kg body weight (by gavage) for four wks [17]. Animals in Group IV were given a combined treatment of copper sulphate as well as purslane, similarly to Group II and Group III animals, respectively. Animals from all the groups were sacrificed by cervical dislocation under light ether anesthesia at the end of the study.

Liver and Body Weights

The body weights of the animals in each group were determined using a weighing balance (Camry; Zhongshan Guangdong, China). The liver from each animal was weighed for determination of the liver weight/body weight ratio.

FTIR Studies

The animal's liver were removed and washed with ice chilled saline. FTIR analysis was carried out as described previously [18]. The FTIR spectra were recorded in the range of 450-4000 cm^{-1}.

Copper Levels

The samples were dried in oven at 60 °C for 48 h and ground in an agate pestle and mortar. Then 1 g powdered samples were mixed with 1ml of hydrochloric acid and nitric acid (2:3 v/v). The mixtures kept in the boiling water for 2 h. Then filtered and made the final volume to 50 ml. Estimation of copper was done by atomic absorption.

Statistical Analysis

The statistical significance of the data was determined by using one-way analysis of variance followed by a multiple post hoc test least significant difference with 5% considered significant. The results are represented as mean ±SD.

RESULTS

FTIR Spectral Analysis

The FT-IR analysis of the samples was done and the functional groups associated were determined (Figs. 1-4). The FT-IR spectrum of the control contains nine major

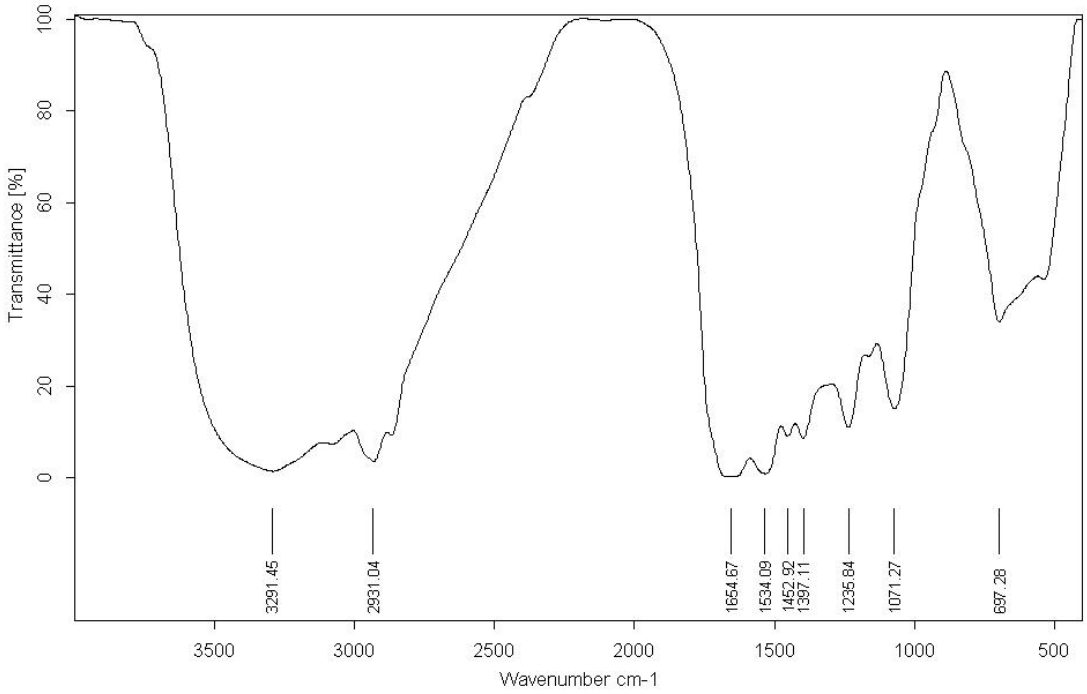

Fig. 1. The FTIR spectra of control liver tissues of rats.

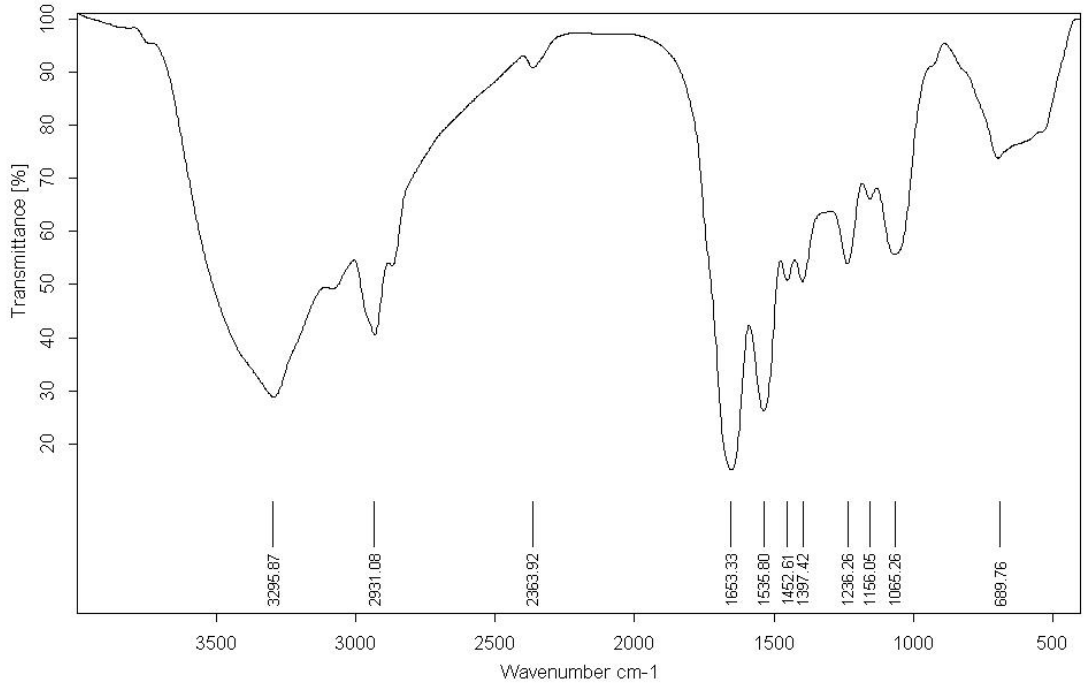

Fig. 2. The FTIR spectra of copper treated liver tissues of rats.

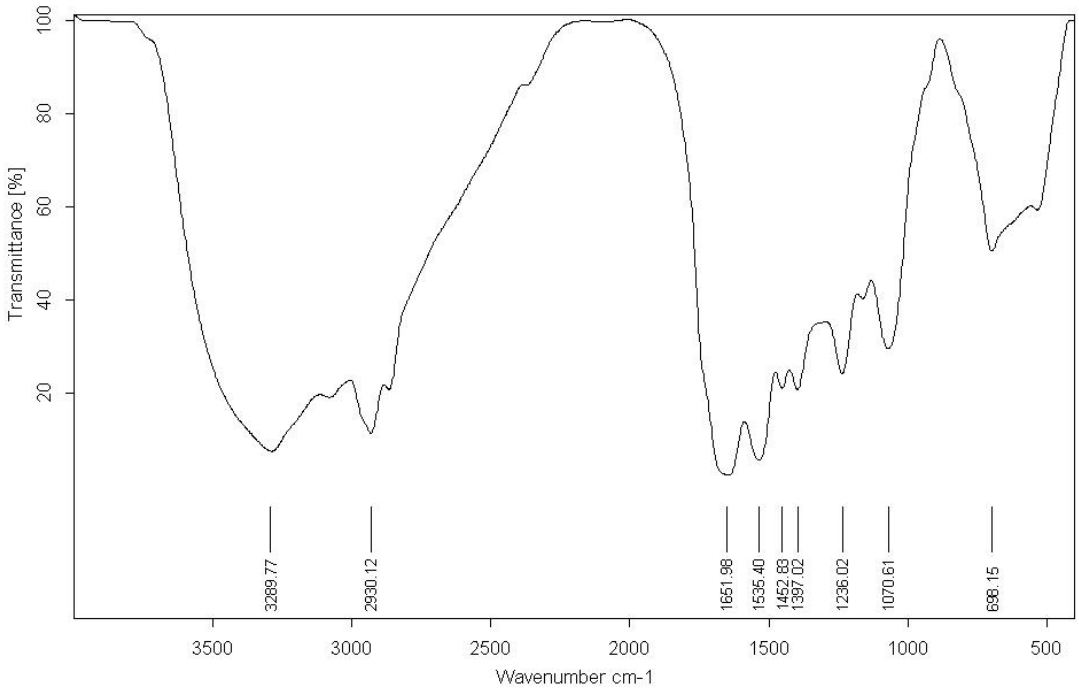

Fig. 3. The FTIR spectra of purslane administrated liver tissues of rats.

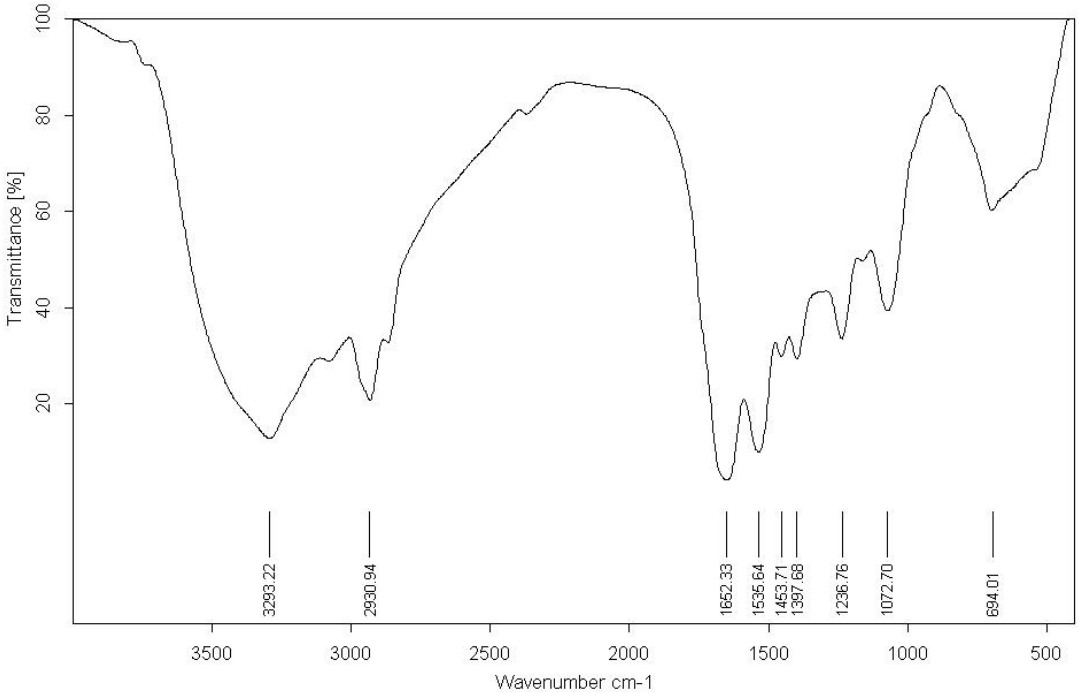

Fig. 4. The FTIR spectra of combined treatments of copper sulphate and purslane liver tissues of rats.

peaks at the range of 3291.45, and 697.28 cm^{-1}: whereas the FT-IR spectrum of the purslane also recorded the same number of peaks lying between 698.15 cm^{-1} and 3289.77 cm^{-1} respectively. Moreover the FT-IR spectrum of the copper sulphate contains 11 major peaks at the range of 3295.87 and 689.76 cm^{-1}: whereas the FT-IR spectrum of the combined treatments of copper sulphate and purslane recorded the nine major peaks lying between 694.01 cm^{-1} and 3293.22 cm^{-1} respectively.

Body Weight

The variation in the body weights of the animals subjected to different treatments are shown in Table 1. It was observed, that the body weights of the normal control and pursalane treated rats increased throughout the study. Copper treatment resulted in a significant decrease ($P < 0.01$) in the body weights when compared to the normal control rats. At end of the respective study, purslane supplementation to copper treated rats were observed to improve ($P < 0.05$) the body weight growth in comparison to animals treated with copper only.

Liver Weights

The variations in the liver weights of the animals subjected to different treatments are shown in Table 1. However, the liver weight gains of the animals treated with copper sulphate was markedly more as compared to the normal controls ($P < 0.001$). Purslane treatment of copper sulphate treated rats tended to decrease ($P < 0.05$) the liver weight in comparison to copper sulphate treated animals.

Liver Weight/Body Weight Ratio

The mean relative liver weights of copper treated rats showed significant increase compared to the control group ($p < 0.001$). Value of the mean relative liver weights (LW/BW ratio) showed a significant decrease ($p < 0.01$) in combined treatment of purslane as well as copper.

Copper Levels

The present study revealed significantly increased ($P < 0.001$) copper levels (Table 2) in the livers of rats following four weeks of copper sulphate treatment. However, supplementation of purslane to copper sulphate treated rats, significantly depressed ($P < 0.05$) the levels of copper when compared to rats treated with copper sulphate alone.

DISCUSSIONS

Purslane is an excellent source of the antioxidant vitamins α-tocopherol, ascorbic acid and β-carotene, as well as glutathione. Purslane is considered as a rich source of many amino acids like isoleucine, leucine, lysine, methionine, cystine, phenylalanine, tyrosine, threonine and valine. Purslane has been described as a “power food of the future” because of its high nutritive and antioxidant properties. Omega-3 fatty acid is a precursor of a specific group of hormones (prostaglandins) and may offer protection against cardiovascular disease, cancers and a number of chronic diseases and conditions throughout the human life. Purslane is a potent antioxidant and is reported to contain omega-3 fatty acids [19].

The toxicity of copper may come about by abnormally increasing the concentration of this ion, since toxicity depends on the concentration of the metal ion [20]. Chronic copper toxicity primarily affects the liver because this is the first site of copper deposition after it enters the blood. Copper toxicity is typically manifested by the development of liver cirrhosis with episodes of hemolysis and damage to other organs [21,22].

Sayre *et al.* (1999) suggested that excessive tissue accumulation of redox-active transition metals (*e.g.*, Cu, Fe) can be cytotoxic, in particular because perturbation in metal homeostasis results in an array of cellular disturbances characterized by oxidative stress and increased free radical production [23]. However, the study of excess copper causing damage to some organs by decreasing antioxidants and increasing lipid peroxidation products may have important implications for the understanding of toxic processes in reproductive diseases. Serving as a cofactor of many enzymes, copper is essential to the life of cells; however, if copper ions are not properly transported, stored, and utilized, redox reactivity leads to the risk of damage to cells and tissues [24]. Previous studies indicate that copper can be metabolized in hepatic tissue and be transferred to metallothionein by GSH thus, the copper overload is reached and depletion of GSH instantaneously results in enhanced cellular toxicity [25]. The present study demonstrates that purslane decrease cu level in liver of

Table 1. Effect of Purslane on Liver Weight (LW), Body Weight (BW) and Liver Weight/ Body Weight (LW/BW) Ratio in Different Treated Animals

Groups	LW (g)	BW (g)	LW/BW ratio (g)
I normal control	6.84 ± 0.59	229 ± 19.38	2.98 ± 0.41
II copper sulphate	9.02 ± 0.79^{c}	189 ± 17.81^{b}	4.92 ± 0.74^{c}
III purslane	6.90 ± 0.85	230 ± 17.67	3.00 ± 0.44
IV copper sulphate + purslane	7.80 ± 0.84^{x}	212 ± 11.51^{x}	3.67 ± 0.52^{y}

$^{c}P < 0.001$ by one-way ANOVA followed by LSD test when values are compared with normal control group; $^{b}P < 0.01$ and $^{x}P < 0.05$ and $^{y}P < 0.01$ by one-way ANOVA followed by LSD test when values of group IV animals are compared with group II animals. Values are expressed as mean ± SD.

Table 2. Copper Levels in Liver of Rats Subjected to Different Treatment

Groups	Copper levels (µg/g tissue)
I normal control	0.88 ±0.42
II copper sulphate	13.63 ± 7/10^{c}
III purslane	0.93 ±0.35
IV copper sulphate + purslane	6.34 ± 6.64^{b,x}

$^{b}P < 0.01$ and $^{c}P < 0.001$ by one-way ANOVA followed by LSD test when values are compared with normal control group; $^{x}P < 0.05$ by one-way ANOVA followed by LSD test when values of

copper treated rats. This indicates that the aqueous purslane extracts either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects.

It has been suggested that one of the most consistent clinical signs indicative of toxicity in animals administered with copper is a reduced growth rate which is accompanied by a fall in body weight [26]. In the present study, a significant decrease in body weight was observed in the experimental groups.These similar to result obtained by Paumen *et al.* [27]. Gharedaash *et al.* (2013) were shown that copper sulphate significantly decreased growth of kutum under chronic sublethal concentration [28]. These result attributed totissue burden of copper, which in turn could cause reduction in the consumption rate, poor food conversion efficiency and increased oxygen consumption rate [29]. Or may attribute to increased metabolic costs and reduced food consumption [30].

In this study the results shows that significant changes in the liver weight of copper treated animals. Reports show that these may be attributedto the accumulation of collagen and extracellular matrix protein in liver tissue [31]. When the liver is damaged, it can initiate regenerative actions [32], thus increasing the weight of liver. Our result is consistent with previous reports that indicated hepatotoxins including thioacetamide induced liver damage by forming free radicals and increase liver weight [33].

In this study it has been noticed that liver has highest Metal Pollution Index therefore it is assumed that high accumulation levels of heavy metals in liver impaired the activity of enzymes which contribute to glycogen synthesis, leading to increase in glycogen content.

The FTIR spectroscopy monitors the vibration modes of functional groups present in proteins, lipids,

polysaccharides, and nucleic acids in liver tissue. Shifts in peak positions indicate the molecular changes associated with macromolecules in a condition. The prime spectral change observed in the present study was of carbohydrate bands. Presence of peaks at 1155 cm^{-1} in the spectra of copper treated rats signified increase in the glycogen contents. Moreover, presence of peak at 2363 cm^{-1} in the spectra of copper treated rats as compared to controls proved changes associated with C = X stretching [34].

The bands in 3,291 cm^{-1} region arise from N-H and O-H stretching modes of proteins, and intermolecular hydrogen bonding. In the experiment, Amide A appeared at 3,291.45 cm^{-1} in control and the band frequency was shift (3295.87 cm^{-1}) in copper-treated rats, whereas the purslane treatment protects the changes and this indicates that the intra molecular hydrogen bonding in proteins of liver tissue maybe disturbed in the copper treated rats. The two prominent absorptions at 1654 cm^{-1} and 1534 cm^{-1} arise from C–O stretching (amide I) and N–H bending (amide II) vibrations of the protein peptide groups [35]. The bands at 1235 cm^{-1} and 1071 cm^{-1} are respectively due to asymmetric and symmetric stretching modes of phosphodiester groups in nucleic acids [36]. However, there was no significant difference in the frequency value of the PO_2^- asymmetric stretching band at 1235 cm^{-1} between the control and treatment groups. The decrease in the area of 1071 band implies a decrease in the relative content of the nucleic acids in the copper treated rats. This effect might be due to the oxidative stress generated during copper poisoning condition as previously reported [37]. Purslane treated group was brought back the shifted peak to control level. This effect was protected by purslane treated group indicates its antioxidant potential against oxidative stress generated during copper toxicity.

The band at 1452 cm^{-1} is mainly assigned to the CH_2 bending mode of lipids [38]. The position of these bands provides information about the order/disorder state of lipids. As seen from Fig. 2 a decrease was observed at 1452 cm^{-1} in copper treated rats. The above effect may directly indicate the antioxidant potential of purslane, which actively scavenge free radicals and activate the defense system through which it protects the macromolecular damage and liver dysfunction. Previous studies have shown that, natural products have promising strategy to protect tissue from oxidative stress and lipid peroxidation under various pathological conditions [39-41]. Consistent with the previous studies [42,43], this work also proven that natural molecules enhances the activity of enzymatic antioxidants thereby protects the tissue against oxidative stress.

CONCLUSIONS

In conclusion, the present study shows that the liver tissues are vulnerable to copper toxicities. High levels of copper in the liver cause oxidative stress which induces significant alteration on the major biochemical constituents such as lipids, proteins and nucleic acids, which can be easily evidenced by FTIR spectroscopy. The study clearly showed that the administration of purslane appreciably attenuates the copper-induced alterations in the FTIR spectra as well as changes in the levels of copper in the liver of copper-treated rats. This finding helps in further research in the investigation of their antioxidant activity and it also useful to utilize of these plants as a source food and medicine.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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