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The inhibitory effect of *Zizypus jujba* fruit growing in Azad Kashmir against lipid peroxidation in mice liver and brain homogenates

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Abstract
The present study was aimed to evaluate the inhibitory effect of *Zizypus jujuba* fruit against lipid peroxidation in mice brain and liver homogenates. The antioxidant activity was performed by lipid peroxidation assay while, total phenolic and flavonoid contents were determined spectrophotometrically. The hot water extract prepared from fruit showed significant ($P<0.05$) inhibition against lipid peroxidation in mice brain and liver homogenates. The high antioxidant activity of *Zizypus jujuba* is due the presence of high content of phenolic and flavonoids. The results suggest that different accessions of *Zizypus jujuba* are potential source of antioxidants and have therapeutic effect of disease induced by oxidative stress.

Key words: *Zizypus jujuba*; Antioxidant activity; Lipid peroxidation; Phenolics; Flavonoids.

Introduction

Oxidative stress is the main cause of biomolecules damage in human body that results in lipid peroxidation, injury to cells, impairment of tissues and gene mutation. Free radicals are continuously produced in cells and causes different diseases which include aging, cardiovascular disorders, cancer, neurodegenerative diseases and inflammation (Pham-Huy, He, & Pham-Huy, 2008). The food is spoiled rapidly during processing and storage when lipid peroxidation is enhanced (Donnelly & Robinson, 1995). These days the studies of natural antioxidant are popular due to their high therapeutic and nutritive values. *Ziziphus Jujuba* belongs to Rhamnaceae family and the Ziziphus genus. *Zizpus jujuba* fruit posses' cytotoxic, antitermite and insecticidal activities (Ahmad, Khan, Bashir, Azam, & Ali, 2011). Triterpenic acids, saponins and flavonoids were detected the leaves of Ziziphus species (Guo et al., 2011). *Zizyphus Jujuba* protects against the seizure and reduces the impairment in cognition (Hwang et al., 2011). Previously, the health-promoting components of a few jujube genotypes have been reported (Akbolat, Ertekin, Menges, Ekinci, & Erdal, 2008). However, more detailed information on the anti-lipid peroxidative properties of additional jujube genotypes could lead to a better understanding and appreciation of the pharmaceutical, nutraceutical, and medicinal value these fruits offer, and an increased consumption of the fruit by the general public. Thus, the objective of this study was to determine the antioxidant capacity and total phenolic content of a number of select jujube fruit accessions collected locally from a diverse ecotype.

Material and methods

Mature fruits *Zizypus jujuba* were collected from district Poonch, Azad Kashmir. The plant specimens were brought to the laboratory in polyethylene bags. Fruits were identified by a botanist at University of Poonch, Rawalakot. Fruits were dried in an oven at 45^{o}C and to a mesh size of 30 mm. For hot water extraction, ground plant material (10 g) was soaked in hot water (250 mL) for 30 minutes and later on filtered. The extraction was carried out thrice and the whole extract was dried in a rotary evaporator at 50 ^{o}C.

Production of TBARS from liver and brain tissues

The TBARS assay was used by a modified method (Ohkawa, Ohishi, & Yagi, 1979). The liver and brain of mice was homogneized in TRIS-HCl (P^{H} 7.4). The homogenate was centrifuged at low speed. The homogenates (100 µl) were incubated with or without 50 µl of Fe (II) and different concentrations of the extracts together with deionized water to give a total volume of 300 µl at 37 ^{0}C for 1 h. The color reaction was done by adding 200µl of 8.1% sodium dodecyl sulphate, 500µl of acetic acid (pH 3.4) and 500 µl of 0.6% TBA, respectively. A standard curve was prepared by using different concentrations of standard MDA, were incubated at 97 ^{0}C for 1 h. The absorbance was read at 532 nm in a spectrophotometer.

Determination of Phenolics Content

The total phenolics content as gallic acid equivalent was determined by Singleton et al., (2015). The aqueous extract (0.5 mL) was added to 2.5 mL, 10% Folin-Ciocalteau's reagent (v/v) and 2 mL of 7.5% sodium carbonate.The reaction mixture was incubated at 45°C for 40 min. and the absorbance was measured at 765 nm in the spectrophotometer. Gallic acid was used as a standard phenol. The mean of three readings was used and the total phenolic content was expressed as milligrams of gallic acid equivalents/g extract.

Determination of total flavonoids

The total flavonoid content as quercetin equivalents/g extract was based on the method reported by [Kosalec-2004] (Kosalec, Bakmaz, Pepeljnjak, & Vladimir-Knezevic, 2004).

Satistical Analysis

The results were expressed as means±SD. Different group means were compared by Duncan Multiple Range Test (DMR) for significance of data.

Results and Discussion

Oxidative stress is now recognized to be associated with more than 200 diseases, as well as with the normal aging process (Ghazanfari et al., 2006). There is a strong correlation between TBARS as a marker of lipid peroxidation and products that reflect oxidative damage to DNA (Chen, Wu, & Huang, 2005). It is known that metal-catalyzed generation of ROS results in an attack not only on DNA and proteins, but also on other cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation. Lipid peroxidation in mice liver and brain homogenate was induced with iron and the potential antioxidant effect of aqueous extract of plant was determined. Table-1 shows the changes in TBARS formation as a result of lipid peroxidation in brain homogenate in the presence and absence of different concentrations of *Zizypus Jujuba*. The Table shows the effect of three accessions of *Zizpus jujuba* fruit on lipid peroxidation. It can be seen that peroxidation increased by treatment with iron, but in the presence of *Zizypus Jujuba* extract it decreased, suggesting inhibition of hydroxyl free radical induced lipid peroxidation by the extract.

Table 1: Inhibitory effect of *Zizypus jujuba* against lipid peroxidation in mice brain homogenate

Treatments	MDA (nmol/g.tissue) accession 1	MDA (nmol/g.tissue) Acession 2	MDA(nomol/g.tissue) Acession 3
Basal	155.37±0.67^{a}	134.89±0.127^{a}	178.87±0.135^{a}
Control	385.06±10.7^{b}	406.88±4.1^{b}	435±4.5^{b}
25 µg/ml	350±2.5^{c}	375±4.5^{c}	367±6.7^{c}
50 µg/ml	325±4.5^{d}	355±5.1^{d}	350±4.5^{d}
75 µg/ml	255±5.6^{e}	315±6.7^{e}	315±4.6^{e}
100 µg/ml	215±6.6^{f}	250±5.6^{f}	250±4.9^{f}
200 µg/ml	185±3.4^{g}	210±4.5^{g}	185±5.6^{g}

The values in Table followed by different letters are significantly different by DMR test.

The changes in TBARS formation as a result of lipid peroxidation in liver homogenate in the presence and absence of different concentrations of *Zizypus jujba*. The lipid peroxidation was stimulated in control compared to the basal indicating the prooxidant behavior of iron. However, the fruit of *Zizypus jujuba* decreased the lipid peroxidation in all treatments and brought close to the basal [Table-2]. Rats overloaded with iron showed toxic effects such as hepatocellular hypertrophy, cardiomyopathy, pancreatic atrophy, splenic white pulp atrophy, and hemosiderosis in the liver, heart, pancreas and endocrine glands, respectively (Berlett & Stadtman, 1997).

Table 2: Inhibitory effect of *Zizypus jujuba* against lipid peroxidation in mice brain homogenate.

Treatments	MDA (nmol/g.tissue) accession 1	MDA (nmol/g.tissue) Acession 2	MDA(nomol/g.tissue) Acession 3
Basal	185.37±0.67[a]	168.89±0.127[a]	186.87±0.135[a]
Control	684±10.7[b]	413.88±4.6[b]	337±4.5[b]
25 µg/ml	634.8±3.5[c]	371±4.5[c]	315.2±6.7[c]
50 µg/ml	590±6.7[d]	318±5.1[d]	271.7±4.5[d]
75 µg/ml	554.4±0.060[e]	285±6.9[e]	250±5.6[e]
100 µg/ml	489.1±0.230[f]	255±5.6[f]	185±4.9[f]
200 µg/ml	455±3.4[g]	215±4.5[g]	167±5.9[g]

The values in Table followed by different letters are significantly different by DMR test.

The phytochemical analysis of *Zizypus jujuba* has shown the high content of total phenolic and flavonoid contents. The mean value of total phenolic content of aqueous extract was 25 mg/g and flavonoid content was 5 mg/g of water extract [Table-3]. The difference of antioxidant activity of different crude extracts depends on the total amount of phenols, flavonoids and aromatic compounds present. This finding indicates that *Z. jujuba* crude extracts are a good potential source of secondary metabolite compounds, which can be used as natural antioxidants against free radical oxidative damage in the human body. This result is in significant agreement with the reported antioxidant activity value of organic crude extracts of *Z. jujuba* described by [Al-Reza-2009] (Al-Reza, Bajpai, & Kang, 2009).

Table 3: Quantity of Phenolics and flavonoids in hot water extract of *Zizypus jujuba*

No.	Phenolics (mg/g)	Flavonoids (mg/g)
1	25±1.4	5±0.1

Conclusions

On the basis of these results it can be concluded that the *Zizpus jujuba* genotypes growing in Azad Kashmir, Pakistan are rich source of secondary metabolites and have effect against lipid peroxidation in mice tissues. The inhibitory effect against lipid peroxidation will additionally benefit in preventing the diseases arising from enhanced lipid peroxidation. More detailed phytochemical characterization of the extract is required to know the active compounds involved in inhibitory activity against oxidative stress.

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