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Research Article

Antioxidant activity and lipid peroxidation of three populations of *ber* (*Ziziphus spinosa*) from Rawlakot, Trarkhel and Balooch

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ABSTRACT

Ber (*Ziziphusspinosa*) is a kind of traditional Chinese medicinal plant, family *Rhamnaceae* distributed in the temperate, tropical and subtropical regions of the world. It can be used as a medicine for mild anxiety, nervousness and sleep-related problems. The study was conducted to evaluate the antioxidant power of *Ziziphusspinosa* whole fruit by *In vitro assay* e.g., Lipid peroxidation. Lipid peroxidation in rat brain homogenate was induced with iron and sodium nitroprusside and the effects of ethanolic extracts of *Z. Spinosa*were determined. There was considerable increase in the progress of Thiobarbituric Acid Reactive Species (TBARS) in rat brain homogenates induced by iron sulphate (10 µM) and (5 µM) SNP. The extracts showed its potential todecrease the development of lipid peroxides in a dose dependent way when test at various concentrations. The results recommend that the fruit of *Z. spinosa*are good source of antioxidant which might be associated with its potential use as a functional food.

Key words: *Ber, Variability, Extracts, Oxidative, Phytoconstitues*

INTRODUCTION

Reactive oxygen species (ROS) are generated spontaneously in body tissues during different metabolism process and are associated with the progression of different degenerative diseases, such as heart diseases, brain stroke, liver damage, rheumatoid arthritis, diabetes, cancer and oxidative stress. Free radicals mutually with other derivatives of oxygen are predictable by products of genetic redox reactions. The powerful toxicity of synthetic antioxidants has aroused interest and scientists have paying attention on isolation of natural antioxidants from natural sources such as spices, herbs, seeds, fruits, cereals and vegetables by extraction, purification and fractionation [2, 6]. *Ziziphus* is a genus containing 135 species of shrubs which are spiny in nature and also including small trees. Its fruits possess considerable levels of antioxidant coup, reducing power, scavenging result on free radicals. Its fruits are used for several other disorders such as tumors and cardiovascular disease associated to the production of radical spices resulting from oxidative pressure. Consequently, medicinal plants can be a potential base of natural antioxidants [8].

Figure 1: The Ber (Fruits) used in the study

The large number of species of *Ziziphus* shows the excellent source of antioxidant and antimicrobial potential. The methanolic extracts of young and mature leaves are rich in their antioxidant phytoconstituents (total phenol, total flavonoid and DPPH scavenging capability, and reducing power). The extracts showed significant antimicrobial activity against diverse micro-organisms. Leaves are of excellent source of natural antioxidant and antimicrobial compounds, and hence can be utilized in herbal innovation [5], Antioxidants from the Ber (fruits) are potential substances, and possessed the ability to defend

the various tissues from the injury caused by free radical which are induced oxidative stress [4]. The present study demonstrated that fruit has an excellent supply of energetic phytochemicals. Presence of adequate and diverse genotype within the area may help to explore the valuable gene pools and select an improved medicinal plant to meet the current demands.

MATERIALS AND METHODS

In vitro assays

The collection of plant material (berries) used in the present study were obtained from diverse areas of Rawalakot, Trarkhel and Balooch during 2014-2015. These berries were kept in plastic pots and transported to the laboratory of Plant Breeding and Molecular Genetics, Faculty of Agriculture, University of Poonch, Rawalakot and were stored to deep freezer at -80°C.

Preparation of plant extract

The mature fruits of the *Ziziphusspinosa*were collected in September 2014 and 2015, from Rawalakot, Trarkhel and Blooch Azad Jammu and Kashmir. The complete fruit was dried in an oven at 40-50°C, then make powdered in a blender and 25 g powder was immersed in 95% ethanol (250 mL) with continuous stirring for 48 h. The extract was filtered by filter paper. Sequential dilutions were prepared to attain the needed concentration of extract for the research. The extract was shifted to dark period at 4°C for more analysis.

Materials

Thiobarbituric Acid (TBA), Malonaldehyde-Bis-Dimethyl Acetal (MDA), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Quercetin, Rutin, Phenanthroline, Sodium Nitroprusside (SNP) and Iron (II).

Laboratory Animal

Locally breed male rats with a mean weight (200±20)g, fed on standard diet and waterwere used for *In vitro* studies. The animals were group-housed (two rats per cage) in controlled room (temperature 2°C and relative humidity 5 %) before carrying out tests or experiment.

Production of Thiobarbituric Acid Reactive Species (TBARS) from animal tissues

Productions of TBARS were resolute via standard method as given by[3]. The rat was deadened with ether. The tissues (brain) was rapidly separated and put on ice. One gram tissues were homogenized in cold 100 mMTris-HCl buffer pH 7.4 (1: 10 w/v) with ten up and down strokes around 1200 rev/min. in a Teflon glass homogenizer. The homogenates were centrifuged for ten minutes at 1400 g to form a bit that was removed and a low-speed supernatant (S1) were used for the experiment. The homogenates (100 µL) were incubated with 50 µL of the different newly arranged oxidants (Iron Sulphate and Sodium Nitroprusside) and various concentrations of the plant extracts at 37°C for 1 h. The tone reaction was conceded out by the addition of 200, 250 and 500 µL each of the 8.1% Sodium Dodecyl Sulphate (SDS), acetic acid (pH 3.4) and 0.6% TBA, correspondingly. The reaction mixtures with serial dilutions of 0.03 mM standard MDA were incubated at 97°C for 1 h. The absorbance was noted at wavelength of 532 nm in a spectrophotometer.

Statistical analysis

The results were expressed as mean ± standard deviation. The data were analyzed statistically by one way ANOVA and different group means were compared by Duncan's multiple range test (DMRT); $p<0.05$ was considered significant in all cases. The software package Statistica was used for analysis of data.

RESULTS

Lipid peroxidation in rat brain homogenate was induced with iron and sodium nitroprusside and the effects of ethanolic extracts of *Z. Spinosa*were determined. Ethnolic extract was taken from three populations (Rawalakot (P1), Trarkhel (P2) and Balooch (P3) of *Z. Spinosa.* There was considerable increase in the progress of TBARS in iron sulphate (10 µM) in induced brain homogenates when compared to the basal or normal (Fig. 2). Ethnolic extracts of *Z. Spinosa* decreased the development of lipid peroxides in a dose dependent way for iron. However, the P3, extract showed better protection against Fe (II) cause lipid peroxidation (97%) at the maximum tested concentration of the extract. Table 1, shows the interaction (inhibition) of the plant extracts with iron induced lipid peroxidation in rat brain. Similarly, the result revealed that incubation of the brain tissue in the presence of 5 µM SNP caused increase in the MDA content of the brain homogenates when compared to the basal or control test in experiment (Fig. 3). However, *Z. Spinosa*caused a significant inhibition of 71% (P1), 63% (P2) and 82 % (P3) in SNP induced lipid peroxidation in the brain tissue when test at various concentrations (Table 2). It is suggested that reactive oxygen species suchas superoxide anion ($O^{-}_{2)}$,hydroxylradical (OH) and nitricoxide inactivates enzymes damage vital cellular components causing tissue damage throughout covalent bindingand lipid peroxidation.

Table 1: Antioxidant activity of ethanolic fruit extract of *Z. Spinosa*(ZS) on iron sulphate induced lipid peroxidation in rat brain homogenate *In vitro*.

Conc. Extract	MDA (µmoL/g.tissue)			MDA inhibition (%)		
	Population 1	Population 2	Population 3	P1	P2	P3
Normal	---	---	---			
Control	---	---	---			
25	204.3±0.136	334.8±0.306	365.2±0.225	84	65	78
50	164.1±0.140	301.1±0.165	239.1±0.236	88	72	93
75	142.4±0.300	234.8±0.280	288±0.267	83	85	87
100	132.6±0.814	281.5±0.092	304.3±0.173	81	76	86
200	129.3±0.246	288.5±0.252	205.4±0.030	80	74	97

Table 2: Antioxidant activity of ethanolic fruit extract of *ZiziphusSpinosa*(ZS) Sodium nitroprusside (SNP) induced lipid peroxidation in rat brain homogenate *In vitro*.

Conc. Extract	MDA (µmoL/g.tissue)			MDA inhibition (%)		
	Population 1	Population 2	Population 3	P 1	P 2	P 3
Normal	---	---	---			
Control	---	---	---			
25	654.4±0.550	413±0.015	337±0.260	51	35	49
50	634.8±0.435	380.4±0.09	315.2±0.106	54	41	54
75	590.2±0.945	282.6±0.130	271.7±0.193	59	59	62
100	554.4±0.060	326.1±0.192	250±0.217	63	51	66
200	489.1±0.230	260.9±0.309	167.4±0.075	71	63	82

Means values and standard deviation (SPSS, following ANOVA).

TBARS Iron induced mice brain Bars with different letters are significantly (p < 0.05) different by DMR test.

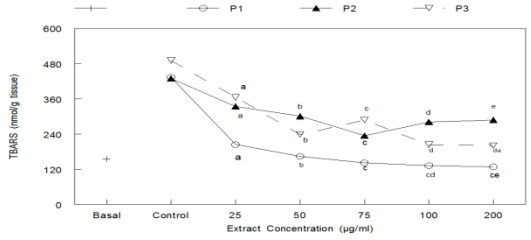

Figure 2: Antioxidant activity of ethanolic fruit extract of *Z. Spinosa*(ZS) on iron sulphate induced lipid peroxidation in rat brain homogenate *In vitro*.

TBARS SNP induced in mice brain Bars with different letters are significantly (p < 0.05) different by DMR test.

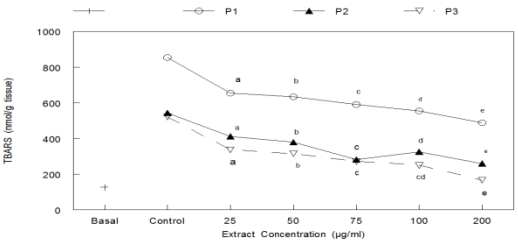

Figure 3: Antioxidant activity of ethanolic fruit extract of *ZiziphusSpinosa*(ZS) Sodium nitroprusside (SNP) induced lipid peroxidation in rat brain homogenate*Invitro*.

DISCUSSIONS

In living systems, free radicals are regularly progressed and they can cause injure to bimolecular tissues and leading to different disease circumstances, particularly degenerative diseases and widespread lysis. Here Fe^{2+} and SNP were used as an implement to arouse lipid peroxidation and they showed significant results. The ethanolic extract of *Z. spinosa*exhibited considerable antioxidant action against two pro-oxidants in tissues. However, in brain it was more efficient against Fe^{2+} induced inhibition compared to SNP increase the formation of TBARS. Increases in the formation of TBARS in Fe^{2+} (10 µM) cause oxidative stress as compared to the basal or control, recommend injure of the tissues with an overload of iron. Rats overloaded with iron showed lethal effects such as hepatocellular hypertrophy, cardio-myopathy, pancreatic atrophy, splenic white pulp atrophy and hemosiderosis in the liver, brain, heart, pancreas and endocrine glands respectively [7]. The protections presented by the *Z. spinosa*suggested that the ethanolic extract might be protecting the brain against toxicities resultant from overload of iron. Sodium nitroprusside is an anti-hypertensive drug that acts via relaxation of vascular smooth muscle and accordingly dilates peripheral arteries and veins. In addition, SNP has been reported to responsible for induction of cytotoxicity throughout the release of cyanide or nitric oxide[1]. The protection existing by *Z.spinosa*extract on brain tissues reported the antioxidant activity of extract and indicates its use in fortuitous intoxications resulting from the overload with SNP.

CONCLUSIONS

In vitro assays indicated that extract of *Z. spinosa* has a potential source of antioxidants, which might be useful in preventing the progress of various oxidative stresses

RECOMMENDATIONS

These findings could result in the development of more valuable and selective new medications from functional foods that are capable of blocking the action of reactive oxygen species involved in oxidative stress.

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