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Investigation of organs allocation and shift of ascorbate radicals levels in different organs by *Cynara scolymus* Linn. extracts - real time electron paramagnetic resonance study

ABSTRACT

A great number of spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant properties. *Cynara scolymus* Linn. plant, cultivated in the Mediterranean regions and central Europe also known as globe artichoke is a perennial, frost sensitive, thistle-like plant belongs to the family Compositae, demonstrated exellent *in vitro*/ *ex vivo* antioxidant properties and strong radical scavenging capacity. By the present study for the first time using EPR spectroscopy we report our investigations on the ''real time'' level of ascorbate radicals and compound- allocation in the livers and in the kidneys of mice after administration of extract from seeds of Bulgarian *Cynara scolymus* Linn. White non-inbred laboratory mice were inoculated i.p.: first group with *Cynara scolymus* Linn. (40 mg/kg) and the second control group was inoculated with the solvent only. The mice were dissected at the different time intervals (10 min – 24 hrs) post injection and fresh tissues from liver and kidneys were homogenated in cold PBS solution and tested for organ allocation. Tissue homogenates in DMSO was prepared for determination of the ascorbate radicals ($Asc^{\cdot}$) level. Maximum concentrations in livers and kidneys were established at 60^{th} min after administration of *Cynara scolymus* Linn. In tested group with *Cynara scolymus* Linn., the level of ascorbate radicals in the livers and in the kidneys were lower than that measured in the control group. In conclusion, these first Electron Paramagnetic Resonance investigations characterize Bulgarian *Cynara scolymus* Linn. as a promising natural antioxidant.

Key words: *Cynara scolymus* Linn., natural antioxidant, EPR spin trapping technique, organs allocation, oxidative biomarkers

Introduction

During recent years, has been much interest in the antioxidant activity of naturally occurring substances (Sgherri et al., 2011). A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant properties.

Cynara scolymus Linn., widely cultivated in the central Europe also known as artichoke is a frost sensitive, thistle-like plant with edible flower buds belongs to the family Compositae (Jiménez- Escrig et al., 2003). Artichoke is found to contain high amounts of flavone glycosides, phytosterol, tannins, and sugars (Fleming, 1998; Ceccarelli et al., 2010), volantile oils (Hammouda, 1993) and bitter sesquiterpene principles (Gebhardt, 2001). *In vitro* study determined that the plant substances (cynaroside, aglycone, luteolin) inhibit cholesterol biosynthesis (Brown & Rice-Evans, 1998; Gebhardt, 1998; Gebhardt, 2001). The artichoke flavonoid luteolin, exhibits good antioxidant properties (Brown & Rice-Evans, 1998; Wang et al., 2003). Chemical and biological diversity of aromatic and medicinal plants depending on such factors, as cultivation area, climatic conditions, vegetation phase, genetic modifications and others is an important impetus to study flora presenting in different growing sites, countries and geographical zones (Llorach et al., 2002; Adzet

et al., 1987; Miliauskasa et al., 2004). In most cases the use is validated by large amounts of literature data that refer to a sinfle plant or extract (Menghini et al., 2010). In previous studies have been demonstrated and concluded that the percent of DPPH radical scavenging capacity of seeds extract of *Cynara scolymus* Linn. determined by spectrophotometry or EPR spectroscopy methods was higher than that of the leaves extract of the plant (Georgieva et al., 2012; Zheleva, 2012). State concentration of $Asc^{\cdot}$ can be used as a measure of oxidative stress in chemical, biochemical, and biological systems (Buettner & Jurkiewicz, 1993).

By the present research, using direct *ex vivo* EPR spectroscopy we report for the first time our studies on the levels of $Asc^{\cdot}$ in livers and kidneys of mice after their treatment by *Cynara scolymus* Linn. seeds extract and its allocation in the same organs.

Materials and Methods

Plant material, chemicals and preparation of plant extract

Cynara scolymus Linn. seeds were provided by Agricultural Faculty, Trakia University, Stara Zagora, Bulgaria.

Ethanol (98%) was purchased by Sigma Chemical Co, St. Louis, USA. Dimethyl sylfoxide (DMSO) was purchased from Sigma Chemical Co, St. Louis, USA and all the other chemicals used in this study were with analytical grade.

100 mg *Cynara scolymus* Linn., the air dried seeds were powdered and stirred in 10 ml of EtOH (70 %) for 5 h at 60°C. After filtration, the extract was brought to a dry substance and kept at 4-8^{o}C in the refrigerator.

Animals

Female white non-inbred mice weighting 26-31g were used. The mice were housed in polycarbonate cages in controlled conditions (12 h light/dark cycles), temperature of 18–23^{o}C and humidity of 40–70%, with free access to tap water and standard laboratory chow. The animals were separated into 2 groups (3/ per group). The first group of mice was injected with saline solution *Cynara scolymus* Linn. seeds extract by a single i.p. injection at a dose of 40 mg/ kg. The control group was inoculated with the solvent only. Experiments were carried out in accordance with national regulations and the European directive 210/63/EU from 22.09.2010, concerning the protection of animals used for scientific and experimental purposes. At different time intervals (10, 30, 60, 90 min, 4th h and 24th h) all animals in the tested and control group were exsanguinated under light ether anesthesia and the livers and kidneys were immediately collected, washed in cool saline and start to prepare tissues homogenates.

Electron Paramagnetic Resonance measurements

For all EPR measurements an X-band EMX^{micro}, EPR spectrometer (Bruker, Germany) equipped with standard Resonator was used. Quartz capillaries were used as sample tubes. The capillary tubes were sealed and placed inside a standard EPR quartz tube (i.d. 3 mm, length 150 mm, wall thickness 0.1 mm) that was placed in the EPR cavity. All EPR experiments were performed in triplicate and repeated at room temperature (18^{o}C - 23^{o}C). Spectral processing was performed using Bruker WIN-EPR and SimFonia software.

Ex vivo EPR investigations of ascorbate radical levels in organ homogenates

The $Asc^{\cdot}$ levels in organ homogenates were studied according to Buettner & Jurkiewicz (1993) with modifications by Zheleva et al. (2011)**.** Samples were weighed and homogenized in DMSO (10% w/v) and centrifuged at 4000g, at 4°C for 10 min. Supernatants were collected and their EPR spectra were recorded. EPR settings were as follows: center field 3505G; sweep width 30G; microwave power 12.96 mw; receiver gain 1x10^{4}; mod. amplitude 5.00G; time constant 327.68 ms; sweep time 82.92 s; 1 scans per sample. The levels of the $Asc^{\cdot}$ were calculated by double integration of the corresponding EPR spectrum and expressed in arbitrary units.

EPR study in the organs allocation of Cynara scolymus Linn. on mice

EPR biodistribution study on *Cynara scolymus* Linn. extract in liver and kidneys was evaluated as previously described by Gadjeva & Coldamova (1994). Plant seeds extract was administrated i.p. and at different time intervals (10, 30, 60, 90 min, 4th h and 24th h) mice livers and kidneys were isolated and washed in cool saline. Tissue samples were immediately homogenized in PBS (10% w/v) and centrifuged at 2000g for 15 min. Supernatants were collected and their EPR spectra were recorded. EPR settings were as follow: center field 3505G; sweep width 70G; microwave power; 13.02 mW; receiver gain 2x10^{4}; mod. amplitude 10G; time constant 327.68 ms; sweep time 327.68 s, 1 scan per sample. Biodistribution levels of *Cynara scolymus* Linn. seeds extract was evaluated on the base of the intensity of the stable EPR singlet signal (Georgieva et al., 2012) registered in the studied supernatants.

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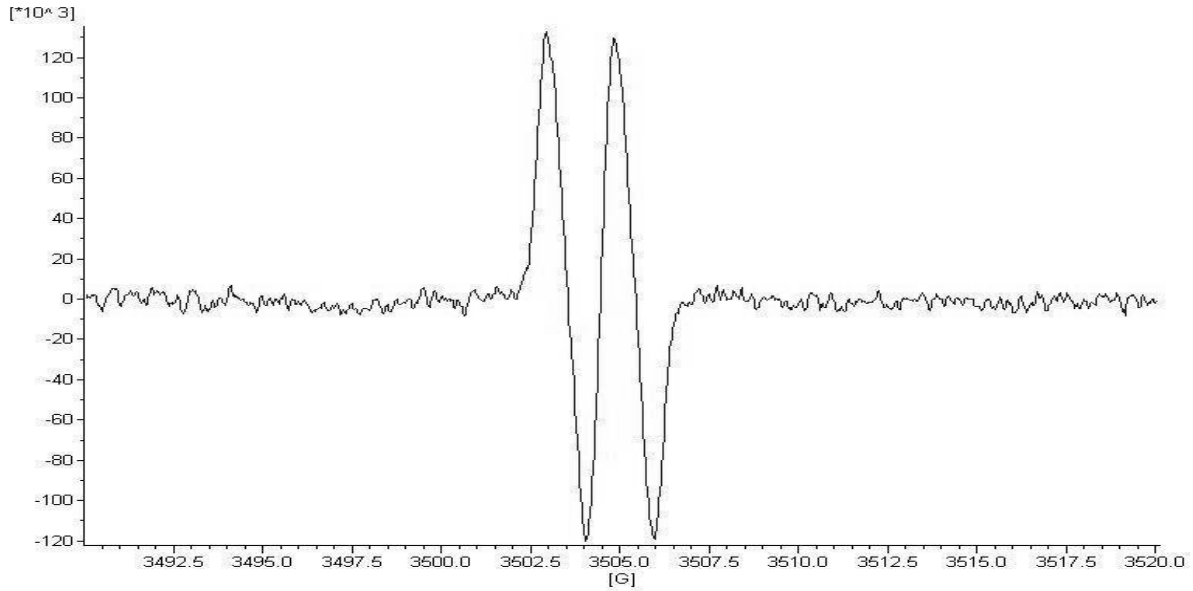

Figure 1. *EPR spectrum of an ascorbate radical registered in tissues homogenates of Cynara scolymus Linn. treated animals.*

Statistical analysis

Statistical analysis was performed with Statistica 8.0, StaSoft, Inc. and results were expressed as means ± standard error (SE). A value of $p < 0.05$ was considered statistically significant.

Results

A strong doublet EPR signal with a g-value of 2.0071±0.0002, typical for the Asc˙radicals, was registered in organ homogenates (Figure 1).

Results from the EPR *ex vivo* studies on the levels of Asc˙ registered in the livers and kidneys of *Cynara scolymus L.* treated animals are shown on Figure 2 and Figure 3.

The levels of Asc˙ in the livers (Figure 2) and in the kidneys (Figure 3) homogenates of animals treated with *Cynara scolymus* Linn. were statistically lower that the same calculated in the control group for any time interval.

Maximum concentrations of the Asc˙ in livers and kidneys homogenates were detected at the 10th min p.i., in both organs.

As is seen 60 min after treatment, the levels of Asc˙ dramatically decreased in the treated mice comparing to the controls (Figures 2 and 3).

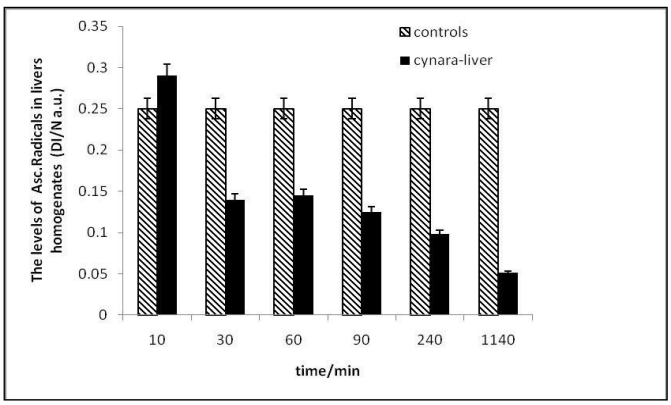

Figure 2. *Levels of ascorbate radicals in liver homogenates of Cynara scolymus Linn. treated animals and controls.*

Organ allocation of *Cynara scolymus* Linn. was evaluated on the base of EPR singlet signal intensity (Georgieva et al., 2012) registered in the tissue homogenates (Figure 4).

As is seen at the 4th h after treatment almost no drug concentration, was found in the studied organs. Maximal intensity of the EPR singlet signal in livers and kidneys were established at 60th min after drug administration (Figure 4). As a whole a higher level of accumulation of *Cynara scolymus* Linn. extract was found in the kidneys comparing to that in the livers for the followed period of the experiment.

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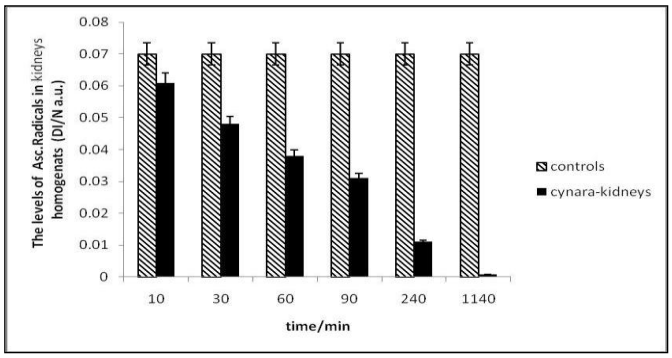

Figure 3. *Levels of ascorbate radicals in kidneys homogenates of Cynara scolymus Linn. treated animals and controls.*

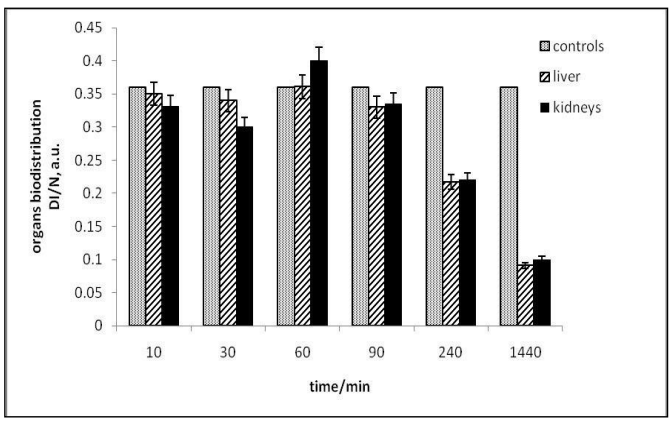

Figure 4. *Real time ex vivo EPR biodistribution of Cynara scolymus Linn. in the liver and kidneys tissue homogenates and controls.*

Discussion

Plants materials contain a wide variety of free radical scavenging molecules, such as flavonoids, anthocyanins, vitamins and endogenous metabolites which are natural products with antioxidant activities (Willett et al., 1995; Visioli et al., 2004). Generally they are used in folk medicine against common colds, asthma, kidney problems, liver diseases and hypertension and to reduce the negative effects of the ROS production.

Oxidative stress induced by different factors generates free radicals, that cause changes in the levels of oxidative stress biomarkers and in progression of oxidative damages (Zheleva, 2012). As a reducing agent and radical scavenger Asc˙ provides effective protection against reactive oxygen species (Halliwell & Gutteridge, 1999). Intensity of Asc˙ EPR signal has been used as a real time indicator of oxidative stress *in vitro* and *in vivo* (Sharma, 1994).

By *in vitro* spectrofotometrical and *ex vivo* EPR studies have been demonstrated that *Cynara scolymus* Linn. seeds and leaves extracts exhibit free-radical scavenging abilities and antioxidant properties (Georgieva et al., 2012; Zheleva, 2012). Lower levels of Asc˙ registered in both organs in comparison with those of the controls (Figures 3 and 4) means that *Cynara scolymus* Linn. extract behaves as an antioxidant at our *ex vivo* experimental conditions.

Conclusion

Based on these preliminary EPR studies and some of formerly reported studies, we consider that *Cynara scolymus* Linn. might find application as an antioxidant and potential organs protector. Further more detailed Electron Paramagnetic Resonance experiments are in progress in our laboratory.

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