Trends in Medical Research

ISSN 1819-3587

Academic Journals Inc.

www.academicjournals.com

Trends in Medical Research 1 (1): 1-8, 2006
ISSN 1819-3587

Study of Some Antioxidant Parameters in Mice Livers Affected with *Urtica pilulifera* Extracts

[1]A.H. Mahmoud, [2]H.M. Motawa, [3]H.E. Wahba and [3]A.Y. Ebrahim
[1]Department of Medicinal Chemistry, National Research Center, Egypt
[2]Department of Pharmacognosea, National Research Center, Egypt
[3]Department of Cultivation and Production of Medicinal and Aromatic Plants, National Research Center, Egypt

Abstract: *Urtica pillulifera* extracts were found to exhibit an antioxidant effect on mice. The effect of two doses of aqueous methanolic extract and other two doses of petroleum ether extract of different plant parts was studied in liver homogenates of the animals. The parameters studied were protein, glutathione reduced form, lipid peroxidation level as well as the activities of Glutathione Transferase (GST), Glutathione Reductase (GR), Glutathione Peroxidase (GSHPx) and Superoxide Dismutase (SOD). Methanolic extracts induced greater effect on the measured antioxidant parameters. Among all plant parts the methanolic extract of the herb showed the best effect; where the antioxidant activity enzymes were elevated and the lipid peroxidation was decreased. In conclusion, *Urtica pilulifera* can be used as natural antioxidant, as a possible food supplement or used in pharmaceutical industry.

Key words: Urtica, medicinal plant extracts, antioxidant

Introduction

There is an increasing interest towards medical plants and their active ingradients since 1980's. Some of the underlying motives can be listed as follows; countries lacking developed chemical industries are searching for affordable treatment by using their own plant sources, synthetic drugs show off their dangerous side effects by time but medical plants have generally centuries-long use and little side effects, many commonly used drugs such as steroidal compounds and ergot alkaloids can be purified from plants much more economically compared to synthetic production. Medicinal plants have multiple actions whereas synthetic drugs have usually only one main effect and additional drugs like vitamins are usually needed to prevent side effects of synthetic drugs. Plant-derived drugs do not necessiate such polypharmacy (Aboolenein, 1982).

Urtica pilulifera (*UP*) is a good plant which is classified as popular plant found in the Palestinian area and in Sinai. Based on number of informants who reported the use of this plant, it can be considered as most important plant. Randal *et al*. (1999) tested the toxicity of the plant and found that petroleum ether extracted from leaves and roots was completely non-leathal even at doses reaching 12.8 mg kg^{-1} and considered non toxic, *UP* dose not have any mutagenic and embyreogenic effects (Graf *et al*., 1994).

Corresponding Author: A.H. Mahmoud, Department of Medicinal Chemistry, National Research Center, Egypt

Urtica sp. have a long history of use in the home as an herbal remedy. A tea made from the leaves has traditionally been used as a tonic and blood purifier. The whole plant is antiasthmatic, antidandruff, astringent, depurative, diuretic, galactogogue, haemostatic, hypoglycaemic and a stimulating tonic (Delcourt *et al*., 1996). An infusion of the plant is very valuable in stemming internal bleeding, it is also used to treat anaemia, excessive menstruation, haemorrhoids and arthritis. In addition, this herb is used to treat rheumatic pain and for colds and cough (Styprekowska and Bieganska, 1980) and skin complaints, especially eczema. The plant is best harvested in May or June as it is coming into flower and dried for later use for medicinal purposes.

However, antioxidant supplements or foods rich in antioxidants may be used to help the human body in reducing oxidative damage by free radicals and active oxygen (Gülçin *et al*., 2002). Recently, various natural products and their effects on health, especially the suppression of active oxygen species by natural antioxidants from teas, spices and herbs, have been intensively studied (Ho *et al*., 1994). Therefore, the development and utilization of more effective antioxidants of natural origin are desired for health (Özen and Korkmaz, 2003).

Aqueous infusions of Mediterranean herbs including *Urtica* species, *ex. U. dioica*, exhibit antioxidant activity towards iron-promoted oxidation of phospholipids, linoleic acid and deoxyribose (Moron *et al*., 1979). It was reported that *Urtica dioica* prevent the damage of rat liver tissue structure (Laurnet, 1981). *Urtica dioica* herbs are used to treat stomachache in Turkish folk medicine (Grice, 1986) and is used against liver insufficiency (Kanter *et al*., 2003). Several different principles, including glucopyranosides (Obertreis *et al*., 1996), glycoprotein (Andersen and Wold, 1978), protein (Tita *et al*.,1993), flavonol glycosides, carotenoids (Kudritskaya *et al*., 1986), as well as biologically-active compounds, such as caffeoyl malic acid and caffeoyl quinic acid (Ohkawa *et al*., 1979), essential oils, formic and acetic acid, histamine, tannins, mucilage, vitamins (A, B1, B2, C, K1, folic and pantothesic acids) (Yagi, 1987) have been identified as contributing to the observed medicinal effects of the plant. The leaves, as well as flowers and roots were used extensively for cancer treatment in Turkey and some reports of positive results exist (Yagi, 1987).

The aim of the present study was to investigate antioxidant activity of the *U. pilulifera* different extracts from different plant parts and its potential use in pharmaceutical industries as a chemopreventive agent.

Materials and Methods

Chemicals

All chemicals were purchased from local firms and were of the highest purity.

Preparation of Plants

Three plant parts (herb, roots and seeds) were exhaustively extracted using two solvents; petroleum ether (80-100%) (PE) and 20% aqueous methanolic (AM) extracts; Pet. ether crude extracts prepared by continuous extraction using soxhlet apparatus while methanolic extracts were prepared by soaking with shaking for plant powders of each organ separately, then each extract was filtered to remove cellular residues. The extracts were concentrated under reduced pressure till free solvents. The different residues were used for experiment.

Animals

Male swiss albino mice (8-9 weeks old), bred in the animal house of National Research Center, were used. The animals were maintained under normal conditions (12 h light/dark cycle) and were fed with pelleted standard laboratory mice feed and tap water.

Experimental Procedure

A total of 49 mice were used, divided into the following groups: Group I (n = 7) was injected interpretoneal with saline, daily for 10 days. This group was designated as a control group. Group II, III and IV (each of 7 animals) were treated with 50 ppm (0.25 mL/mouce) of petroleum ether extract of *U. pilulifera* (herb, root and seed) through interpretoneal injection daily for 10 days. Group V, VI and VII (each of 7 animals) were treated with 50 ppm (0.25 mL mouce) of methanolic extract of herb, root and seed through interpretoneal injection daily for 10 days. This treatment is repeated with high concentration (200 ppm).

Preparation of Subcellular Fractions from the Liver

Mice were killed by cervical dislocation. Liver homogenates were prepared in ice-cold bidistilled water. The homogenate was centrifuged at 3000 rpm in cooling centrifuge (-4°C) for 10 min. The supernatant was used for determination of NP-SH and different enzymatic activities.

Determination of NP-SH Group

The level of sulfhydryl groups was determined using 5, 2-dithiobis-2-nitrobenzoic acid (DTNB) according to Neugebauer *et al*. (1995).

Determination of Lipid Peroxidation (LPO)

LPO of liver microsomes was determined spectrophotometrically as the number of nmoles of malondialdehyde (MDA) formed per mg protein at 532 nm (Oktay *et al*., 2003).

Assay of Glutathione S-transferase (GST)

It was assayed using 1-chloro-2, 4- dinitrobenzene (CDNB) as substrate (Habig *et al*., 1974). The substrate was previously dissolved in the least amount of absolute ethyl alcohol. The formation of conjugated derivatives was followed spectrophotometrically at 340 nm as a function of time.

Determination of Protein

Microsomal and cytosolic proteins were determined using the method of Bradford (Bradford, 1976).

Assay of Glutathione Reductase (GR)

GR was assayed spectrophotometrically with slight modification according to Calberg and Mannervik (1974).

Assay of Glutathione Peroxidase (GPHPx)

GSH peroxidase activity was determined as described by Lebedev *et al*. (2001).

Assay of Superoxide Dismutase (SOD)

Measurement of SOD activity was carried out spectrophotometrically (Folhé and Otting, 1984).

Statistical Analysis

Results are expressed as mean±SD. Statistical analysis was determined using ANOVA following Dunnet's t-test. Values showed a significant difference from those of control groups. The SPSS 10.0 (Statistical Program for Social Sciences) was used for statistical analysis (Campbell, 1989).

Results

Antioxidant Activity of Aqueous Methanolic and Petroleum Ether Extracts at 50 ppm

Table 1 and 2 indicate that all six *U. pilulifera* extracts significantly enhanced the protein synthesis in mice liver and enhanced glutathione reductase activity but the glutathione transferase activity reduced than control group using aqueous methanolic extracts of herb and roots. Superoxide dismutase activity was significantly enhanced by using all extracts.

Antioxidant Activity of Aqueous Methanolic Extracts at 200 ppm

All estimated protein concentration and antioxidant parameters were enhanced specially with herbal extract, the protein concentrations measured in liver homogenate of mice treated with 200 ppm aqueous methanolic extracts were enhanced by 25.97, 75.86 and 78.7% for herb, root and seeds, this increment of protein synthesis was in parallel with induction of enzyme levels.

Table 3 shows that, glutathione concentrations determined in mice liver homogenate were significantly increased specially in case of mice treated by herbal extracts (299.2, 84.2 and 22.5% for herb, root and seeds, respectively). Glutathione transferase activity was elevated by 285.76, 118.69 and 181% using herbs, roots and seeds extracts, respectively. The glutathione reductase activity were enhanced with animal treated by herb extract only (36.69%) whereas root and seeds extract were reduced by 24.88 and 20.69%, respectively. Glutathione peroxidase has the same manner which induced by herbal extract (68.3%) and reduced by the other extracts; root and seeds (-40.14 and -43.66%, respectively). The Superoxide Dismutase (SOD) levels were increased by (232.8%) using extract of herb while the other extracts (roots and seeds extracts) caused lowered increments by 31.25 and 64.84%, respectively. The enhancement of antioxidant parameters caused high reduction in lipid

Table 1: Effect of *Urtica pilulifera* methanolic extracts at 50 ppm on antioxidant parameters in mice liver homogenates

Groups	Protein conc (mg g tissue^{-1})	G. Transferase (mMol mg P^{-1} min^{-1})	G. reductase (mMol mg P^{-1} min^{-1})	G. peroxidase (mMol mg P^{-1} min^{-1})	SOD unit (mg P^{-1})	Lipid peroxides (µMol g tissue^{-1})
(1) Control	296.12±0.478	3.65±0.27	3.47±0.11	2.23±0.17	0.68±0.09	19.05±0.61
LSD	(2,3,4)	(2,3,4)	(3)	(2,3)	(2,3,4)	(2,3,4)
(2) Herb ext.	341.61±0.51	3.02±0.08	3.68±0.22	1.34±0.23	3.34±0.19	12.16±0.4
%	15.36	-17.26	6.05	-39.91	391.18	-36.17
LSD	(1)	(1)	(3)	(1,4)	(1,3,4)	(1,4)
(3) Root ext.	365.01±0.12	3.07± 0.12	2.15±0.17	1.57± 0.15	1.51±0.32	13.59±0.2
%	23.26	-15.89	-38.04	-29.6	122.06	-28.66
LSD	(1)	(1)	(1,2,4)	(1,4)	(1,2)	(1)
(4) Seeds ext.	365.01±0.12	2.63±0.19	3.5±0.14	2.53±0.47	1.29±0.3	19.1±0.3
%	23.26	-27.94	0.86	13.45	89.71	0.26
LSD	(1)	(1)	(3)	(2,3)	(1,2)	(1,2)
ANOVA						
F P	144.49 0.00	47.96 0.00	5.92 0.01	28.74 0.00	69.54 0.00	27.36 0.00

Table 2: Effect of *Urtica pilulifera* petroleum ether extracts at 50 ppm on antioxidant parameters in mice liver homogenate

Groups	Protein conc (mg g tissue^{-1})	G. Transferase (mMol mg P^{-1} min^{-1})	G. reductase (mMol mg P^{-1} min^{-1})	G. peroxidase (mMol mg P^{-1} min^{-1})	SOD (mg P^{-1})	Lipid peroxides (µMol g tissue^{-1})
(1) Control	296.12±0.478	3.65±0.27	3.47±.11	2.23±0.17	0.68±0.09	19.05±0.61
LSD	(2,3,4)	(2,3,4)	(3)	(2)	(2,3,4)	(2,3)
(2) Herb ext.	313.66±0.296	2.41±0.12	3.69±0.34	1.48±0.17	1.81±0.21	23.98±0.22
%	5.92	-33.97	6.34	-33.63	166.18	25.88
LSD	(1,3,4)	(1,3)	(3)	(1,3,4)	(1,3,4)	(1,3,4)
(3) Root ext.	350.59±0.443	2.05± 0.12	4.13±0.13	2.4±0.18	1.32±0.04	22.48±046
%	18.39	-43.84	19.02	7.62	94.12	18.01
LSD	(1,2,3)	(1,3)	(3)	(2)	(1,2,4)	(1,2,4)
(4) Seeds ext.	338.56±0.388	2.64±0.23	3.63±0.26	2.18±0.06	1.59±0.04	19.11±1.71
%	14.33	-27.67	4.61	-2.24	133.82	0.31
LSD	(1,2,3)	(1,3)	(3)	(2)	(1,2,3)	(2,3)
ANOVA						
F P	144.49 0.00	47.96 0.00	5.92 0.00	28.74 0.00	69.54 0.00	27.36 0.00

Conc: concentration, G: Glutathione, P: Protein, SOD = Superoxide dismutase, F: ratio of one way ANOVA.
p: value of ANOVA significant <0.05

Table 3: Effect of *Urtica pilulifera* methanolic extracts at 200 ppm on antioxidant parameters in mice liver homogenate

Groups	Protein conc (mg g tissue^{-1})	Glutathione conc. (mg tissue^{-1})	G. Transferase (mMol mg P^{-1} min^{-1})	G. reductase (mMol mg P^{-1} min^{-1})	G. peroxidase (mMol mg P^{-1} min^{-1})	SOD (mg P^{-1})	Lipid peroxides (µMol g tissue^{-1})
(1) Control	282.12±1.4	1.2±0.07	3.37±0.34	4.06±0.63	1.42±0.17	1.28±0.01	14.83±1.12
LSD	(2,3,4)	(4,5,6)	(2,3,4)	(2,3,4)	(2,3,4)	(2,3,4)	(2,3,4)
(2) Herb ext.	355.4±0.41	4.79±0.08	13±0.47	5.55±0.52	2.39±0.01	4.26±0.05	2.70±0.66
%	25.97	299.17	285.76	36.69	68.31	232.81	-81.8
LSD	(1, 3, 4)	(1, 3, 4)	(1, 3, 4)	(1,3,4)	(1,3,4)	(1,3,4)	(1,3,4)
(3) Root ext.	496.1±0.29	2.21±0.17	7.37±0.43	3.05±0.1	0.85±0.01	1.68±0.39	4.47±1.03
%	75.86	84.17	118.69	-24.88	-40.14	31.25	-69.86
LSD	(1,2)	(1,2,4)	(1,2,4)	(1,2)	(1,2)	(1,2,4)	(1,2,4)
(4) Seed ext.	504.1±0.77	1.47±.17	9.47± 1	3.22± 0.32	0.80±0.17	2.11±0.19	6.17±0.66
%	78.68	22.5	181.01	-21.69	-43.66	64.84	-57.39
LSD	(1,2)	(1,2,3)	(1,2,3)	(1,2)	(1,2)	(1,2,3)	(1,2,3)
ANOVA							
F P	170.94 000	509.58 000	170.67 0.00	26.57 0.00	128.53 000	111.81 000	145.49 000

Table 4: Effect of *Urtica pilulifera* petroleum ether extracts at 200 ppm on antioxidant parameters in mice liver homogenate

Groups	Protein conc (mg g tissue^{-1})	Glutathione conc (mg tissue^{-1})	G. Transferase (mMol mg P^{-1} min^{-1})	G. reductase (mMol mg P^{-1} min^{-1})	G. peroxidase (mMol mg P^{-1} min^{-1})	SOD (mg P^{-1})	Lipid peroxides (µMol g tissue^{-1})
(1) Control	185±0.09	2.5±0.41	0.69±0.05	4.89±0.15	2.85±0.19	0.19±0.002	1.25±0.03
LSD	(2,3,4)	(2,3,4)	(1,2)	(2,3,4)	(2,3,4)	(2,3,4)	(2,4)
(2) Herb ext.	430.6±.28	4.17±0.51	1.86±0.19	7.82±0.54	8.2±0.33	0.24±0.004	0.86±0.02
%	132.76	66.8	169.57	59.92	187.72	26.32	-31.2
LSD	(1,3,4)	(1,3,4)	(1,3,4)	(1,3,4)	(1,3,4)	(1,3,4)	(1,3,4)
(3) Root ext.	408.8±0.15	4.5±0.84	1.41±0.34	6.12±0.34	3.82±0.24	0.04±0.002	1.4±0.15
%	120.97	80.0	104.35	25.15	34.04	-78.95	12.0
LSD	(1,2,4)	(1,2,4)	(1,2,4)	(1,2,4)	(1,2,4)	(1,2,4)	(2,4)
(4) Seed ext.	304.7±0.13	5.67±0.52	0.62±0.37	5.39±0.43	3.28±0.49	0.09±0.005	1.91±0.16
%	64.7	126.8	-10.14	10.22	15.09	52.63	52.8
LSD	(1,2,3)	(1,2,3)	(1,2,3)	(1,2,3)	(1,2,3)	(1,2,3)	(1,2,3)
ANOVA							
F P	2671.4 0.00	32.8 0.00	151.24 0.00	495.7 0.00	62.47 0.00	54.36 000	20.05 000

Conc: concentration, G: Glutathione, P: Protein, SOD = Superoxide dismutase, F: ratio of one way ANOVA.
p: value of ANOVA significant <0.05

peroxidation and the highest reduction was caused by aqueous methanolic herb extract which represented 81.8% followed by roots and seeds extract (69.86% and 58.39%, respectively).

Antioxidant Activity of Petroleum Ether Extracts at 200 ppm

Table 4 shows that liver protein concentration in mice treated by petroleum ether extract were significantly increased by 132.76, 120.97 and 64.7% for herb, roots and seeds extracts respectively. The glutathione concentrations were enhanced by 66.8, 80 and 126.8% for herb, roots and seeds extract treatments, at the same time the glutathione transferase activities were induced by 169.57, 104.35% for herb and roots extracts treatments while it decreased by seeds extract treatment (-10.14%). The glutathione reductase activities were enhanced by different levels; 59.92% for herb extract treatment, 25.15% for roots extract treatment and 10.22% with seeds extract treatment. On the other hand, glutathione peroxidase has high significant increase by 187.72% when animals treated by petroleum ether extract of herb, but roots and seeds petroleum ether extract did not have the same effect which caused enhancement by 34.04 and 15.09%, respectively.

Data of superoxide dismutase activity in liver tissue of treated animals showed an increment using herb petroleum ether extract (26.32%) whereas they decreased by 78.95 and 52.63% in animal treated by roots and seeds pet. ether extracts. Herb petroleum ether extract has a strongest effect on antioxidant parameters, it inhibited lipid peroxides production (-31.2%) but the other two extracts did not have the same activity, they enhanced lipid peroxides by 12 and 52.8% for roots and seeds extracts, respectively.

Discussion

In recent years, natural bioactive compounds have been used as chemo preventive agents to inhibit hazardous effects of xenobiotics different sites. Xenobiotic detoxification is controlled by the liver as the main organ.

In this study, application of the petroleum ether and hydro alcoholic extracts of *U. pilulifera* to mice showed a marked effect on protein synthesis, some hepatic biotransformation enzyme systems and antioxidant enzymes. The intraperitoneal injection of *U. pilulifera* extracts (0.735 and 2.94 mg kg^{-1} day^{-1} for 10 days) had discernible effects on the tested parameters.

Protein concentration was increased by different extracts in the order of herb>root >seed by using PE extracts and take the opposite direction by using methanol extracts (Table 1 and 2). This may be due to presence of a procial amount of active compounds that enhance protein synthesis. This process also differs according to content of active compounds in different part of plants which affects some hepatic biotransformation and antioxidant enzymes. The enhancement of protein synthesis is also dose dependent (Table 1 and 3). The decrease of lipid peroxidation by *U. pilulifera* suggests a role of cytoprotection as well as prooxidant and peroxisomes induced membrane dangerous.

The findings are coincided with Özen and Korkmaz (2003) who used hydroalcoholic herb extract of *U. dioica* by 50 mg and 100 mg $kg^{-1}day^{-1}$. Table 1 and 2 showed that *UP* has a high antioxidant activity although it was used in low concentration, this indicate a high reducing power activities of the plant. The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Samur *et al*., 2001).

GST, which plays an important role in conjugating the metabolites resulting from the action of cytochrome P450, with endogenous ligands (reduced glutathione) favoring their elimination from the body of organisms, is a critical detoxification enzyme (Hartman and Shankel, 1990). It has already been reported that glutathione S-transferase induces protection against various cytotoxic, mutagenic and carcinogenic chemicals (Ketterer, 1988). The study showed that GST activity increases (in common) by using different extracts and this increase differs in its magnitude according to dose and parts of plants. The antioxidant enzymes in mice liver is enhanced with using leaf extracts (PE and AM) but decrease in roots and seeds. This may be due to quinching activity of different parts of plants and hydrogen donating ability of separate compounds (Gülçin *et al*., 2004). The phytochemical investigation of the plants showed the presence of flavonoids, coumarins, some sterols, alkaloids and hydrocarbons by aqueous menthanol extract meanwhile sterols, fatty acids and terpenes were extracted by petroleum ether. Flavonoids, phenolic acids and some terpenes possess antioxidant activities in different mechanisms which gave the induction of different antioxidant estimated parameters.

On the basis of the results of this study, it is clearly indicated that *U. pilulifera* has a powerful antioxidant activity against various oxidative systems *in vivo* moreover, it can be used as accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. In addition, free radicals have been demonstrated to be a contributing factor in the tissue injury and modulation of the pain, phenolic compounds which present in plants appear to be responsible for the antioxidant activity of extracts. Some studies have revealed that the antioxidants melatonin and β-carotene potentiate the antinociceptive responses (Ruiz-Larrea, 1997).

References

Aboolenein, A.A., 1982. Back to medicinal plants therapy. Hamdard, 40: 1-4.

Andersen, S. and J.K. Wold, 1978. Water-soluble glycoprotein from Urtica-dioica leaves. Phytochemistry., 17: 1875-1877

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem.,72: 248-254.

Calberg, I. and B. Mannervik, 1974. Purification and characterization of flavoenzyme glutathione reductase from rate liver. J. Biol. Chem., 250 : 5475-5480.

Campbell, R.C., 1989. Statistics for Biolpogists 3rd Edn., Cambridge University Press, Cambridge New York, Melbourne, Sydney.

Delcourt, M., W.J. Peumans, M.C. Wagner and P. Truffa-Bachi, 1996. Specific deletion of mature thymocytes induced by the plant superantigen *Urtica dioica* agglutinine. Cell. Immunol., 168: 158-164.

Folhé, L. and F. Otting, 1984. Superoxide dismutase assays. Methods Enzymol., 105 : 93-104.

Graf, U., A. Moraga, R. Castro and E. Carrillo, 1994. Genotoxicity testing of different types of beverages in the drosophila wing somatic mutation and recombination test. Food Chem. Toxicol., 32: 423-30.

Grice, H.C., 1986: Safety evaluation of Butylated Hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract. Food Chem. Toxicol., 24: 1127-1130.

Gülçin, I., M. Oktay, Ö.I. Küfrevioğlu and A. Aslan, 2002. Determination of antioxidant activity of lichen *Cetraria islandica* (L.). Ach. J. Ethnopharmacol., 79: 325-329.

Gülçin, I., Ö.I Küfrevioğlu, M. Oktay and M.E. Büyükokuroğlu, 2004. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urtica dioica* L.). J. Ethnopharmacol., 90: 205-215.

Habig, V.H., M.J Pabstt and W.B. Jakoby, 1974. Glutathione S-transferase, the first enzymatic step in mercapturic acid formation. J. Biochem., 249: 7130-7139.

Hartman, P.E. and D.W. Shankel, 1990. Antimutagens and anticarcinogens; A survey of putative interceptor molecule. Environ. Mol.Mutagen.,15:145-182.

Ho, T., T. Osawa, M.T. Huang and R.T. Rosen, 1994. Food Phytochemicals for Cancer Prevention. II. Tea, Spices and Herbs. ACS Symposium Series 547, American Chemical Society, Washington, DC, pp: 2-9.

Kanter, M., I. Meral, S. Dede, H. Gunduz, M. Cemek, H. Ozbek and I. Uygan, 2003. Effects of *Nigella sativa* L. and *Urtica dioica* L. on lipid peroxidation, antioxidant enzyme systems and some liver enzymes in CCl4-treatedrats. J. Vet. Med. Physiol. Pathol. Clin. Med., 50: 264-268.

Ketterer, B., 1988. Protective role of glutathione and glutathione-s-transferase in mutagenesis and carcenogenesis. Mut. Res., 202: 343-275.

Kudritskaya, S.E., G.M. Fishman, L.M. Zagorodskaya and D.M. Chikovani, 1986. Carotinoids *Urtica dioica* L. Khimiya Prirodnykh Soedinenii., 5: 640-641.

Laurnet, E., 1981. Edible and medicinal plants. Toxicol., 160: 55-61.

Lebedev, A.A., E.A. Batakov, V.A Kurkin, E.A Lebedeva, G.G. Zapesochnaya, E.V. Avdeeva, G.V. Simonova and A.V. Volotsueva, 2001. The antioxidative activity of a complex hepatoprotective preparation, silybokhol. Rastitel'nye Resursy, 37: 69-75.

Moron, M.S., J.W. Depierre and B. Mannervik, 1979. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochem. Biophys. Acta., 582: 67-78.

Neugebauer, W., P. Winterhalter and P. Schreier, 1995. 3-Hydroxyalpha-ionyl-beta-d-glucopyranosides from stinging nettle (*Urtica dioica* L.) leaves. Nat. Prod. Lett., 6: 177-180.

Obertreis, B., K. Giller, T. Teucher, B. Behnke and H. Schmitz, 1996. Anti-phlogistic effect of *Urtica dioica* folium extract in comparison to caffeoyl malic acid. Arzneimittel Forschung., 46: 52-56.

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-58.

Oktay, M., I. Gülçin and Ö.I. Küfrevioğlu, 2003. Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Lebensmittel-Wissenchaft und Technol., 36: 263-271.

Özen, T. and H. Korkmaz, 2003. Modulatory effect of *Urtica dioica* leaf extract on biotransformation enzyme systems, antioxidant enzymes, lactate dehydrogenase and lipid peroxidation in mice. Ethenopharmacology, 90: 205-215.

Randal, C., K. Mccthanm, H. Randal and F. Dobbs, 1999. *Urtica dioica* for joint pain: An exploratory study of this complementary therapy. Compl. Ther. Med., 7: 126-131.

Ruiz-Larrca, M.B., 1997. Antioxidant activity of phytoestrogenic isoflavones. Free Radical Res., 26: 63-70.

Samur, M., H.S. Bozcuk, A. Kara and B. Savas, 2001. Factors associated with utilization of nonproven cancer therapies in Turkey. Supportive Care in Cancer, 9: 452-458.

Styprekowska, E. and J. Bieganska, 1980. Investigations on an increase of extraction yield of protein from *Urtica dioica* L. leaves. Herba Polonica, 26: 171-176.

Tita, B., P. Facecendini, U. Bello, L. Martinoli and P. Bello, 1993. *Urtica dioica* L: Pharmacological effect of ethanol extract. Pharmacol. Res., 27: 21-23.

Yagi, K., 1987. Lipid peroxides and human disease. Chemistry and Physics of Lipids, 45: 337-341.