The Egyptian Journal of Hospital Medicine Vol., 21: 33 – 42 December 2005
I.S.S.N: 12084
2002–1687

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

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Abstract

Urtica pilulifere 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 levels 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 (*U.P.*) 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 and considered non toxic, *U. P.* does not have any mutagenic and embyreogenic effects (Graf *et al*, 1994).

Urtica sp. has 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, deputive, 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. Externally, the plant is used to treat arthritic pain, gout, sciatica, neuralgia, haemorrhoids and hair problems .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 s 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 glucopyr-anosides (Obertreis et al.,1996), glycoprotein (Andersen, and Wold, 1978), protein (Tita *et al*.,1993), flavonol glycosides, carotenoids (Kudritskaya *et al*.,1986.), as well as biolo-gically 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 pharm-aceutical industries as a chemopreventive agent.

Material and Methods

Chemicals

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

Preparation of plants

Three plant parts (herbs, roots and seeds) were exhaustively extracted using two solvents; petroleum ether (80-100%) (PE) and 20% aqueous methanolic (AM) extracts. P.E. crude extracts prepared by contineous 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 hour 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/ mouse) of petroleum ether extract of *U. pilulifera* (herbs, roots 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 concentretion (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 centifuge (-4° C) for 10 min. The supernatent 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 (GPx)

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 peterolum 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 glutathione reductase activity but the glutathione transferase activity was reduced than control group using aqueous methanolic extracts of herbs and roots. Superoxide dismutase activity was significantly enhanced by using all extracts.

Antioxidant activity of aqueous methanolic extracts at 200 ppm:

All estimated protein conc. 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 herbs, roots and seeds, this increment of protein synthesis was in parallel with induction of enzyme levels.

Table (3) indicates that, glutathione concentrations determined in mice liver homogenate were significantly increased specially in case of mice treated by herbal extracts (299.17, 84.17 and 22.5% for herb, root and seeds respectively). Glutathione

transferase activities was elevated by 285.76 , 118.69 and 181% for herb, root and seed 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 peroxidation and the highest reduction was caused by aqueous methanolic herb extract which represented 81.8% and followed by roots and seeds extract (69.86 and 58.39% respectively).

Antioxidant activity of petroleum ether extracts at 200ppm:

Table (4) indicates that mice treated by petroleum ether extract were significantly increased protein concentrations of liver 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 12.0 and 52.8% for roots and seeds extracts, respectively.

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±.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 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± .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±	2.4± 0.18	1.32± 0.04	22.48± .46
%	18.39	-43.84	0.1319.02	7.62	94.12	18.01
LSD	(1,2,4)	(1,2,4)	(1,2,4)	(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 ismutase
F: ratio of one way ANOVA. P: value of ANOVA significant < 0.05

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

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 Unit mg P^{-1}	Lipid peroxides µMol g tissue^{-1}
(1)control **LSD**	282.12± 1.4 (2, 3, 4)	1.2± 0.07 (4, 5, 6)	3.37± 0.34 (2, 3, 4)	4.06± 0.63 (2, 3, 4)	1.42±0.17 (2,3,4)	1.28±0.01 (2,3,4)	14.83±1.12 (2,3,4)
(2)Herb ext. **%** **LSD**	355.4± 0.41 25.97 (1, 3, 4)	4.79± 0.08 299.17 (1, 3, 4)	13± 0.47 285.76 (1, 3, 4)	5.55± 0.52 36.69 (1, 3, 4)	2.39±0.01 68.31 (1,3,4)	4.26±0.05 232.81 (1,3,4)	2.70±0.66 -81.8 (1,3,4)
(3)Root ext. **%** **LSD**	496.1± 0.29 75.86 (1, 2)	2.21± 0.17 84.17 (1, 2, 4)	7.37± 0.43 118.69 (1, 2, 4)	3.05± 0.1 -24.88 (1, 2)	0.85±0.01 -40.14 (1,2)	1.68±0.39 31.25 (1,2,4)	4.47±1.03 -69.86 (1,2,4)
(4)Seed ext. **%** **LSD**	504.1±0.77 78.68 (1, 2)	1.47± .17 22.5 (1, 2, 3)	9.47± 1 181.01 (1, 2, 3)	3.22± 0.32 -20.69 (1, 2)	0.80±0.17 -43.66 (1,2)	2.11±0.19 64.84 (1,2,3)	6.17±0.66 -58.39 (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 200ppm on antioxidant parameters in mice liver homogenates

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 Unit mg P^{-1}	Lipid peroxides µMol g tissue^{-1}
(1)control **LSD**	185± 0.09 (2, 3, 4)	2.5± 0.41 (2, 3, 4)	0.69± 0.05 (1, 2)	4.89± 0.15 (2, 3, 4)	2.85± 0.19 (2, 3, 4)	0.19±0.002 (2, 3, 4)	1.25±0.03 (2,4)
(2)Herb ext. **%** **LSD**	430.6± .28 132.76 (1, 3, 4)	4.17± 0.51 66.8 (1, 3, 4)	1.86± 0.19 169.57 (1, 3, 4)	7.82± 0.54 59.92 (1, 3, 4)	8.2± 0.33 187.72 (1, 3, 4)	0.24±0.004 26.32 (1, 3, 4)	0.86±0.02 -31.2 (1,3,4)
(3)Root ext. **%** **LSD**	408.8±0.15 120.97 (1, 2, 4)	4.5± 0.84 80.0 (1, 2, 4)	1.41± 0.3 104.35 (1, 2, 4)	6.12± 0.34 25.15 (1, 2, 4)	3.82± 0.24 34.04 (1, 2, 4)	0.04±0.002 -78.95 (1, 2, 4)	1.4±0.15 12.0 (2,4)
(4)Seed ext. **%** **LSD**	304.7±0.13 64.7 (1, 2, 3)	5.67± 0.52126.8 (1, 2, 3)	0.62± 0.37 -10.14 (1, 2, 3)	5.39± 0.43 10.22 (1, 2, 3)	3.28± 0.49 15.09 (1, 2, 3)	0.09±0.00 52.63 (1, 2, 3)	1.91±0.16 52.8 (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

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 *Urtica pilulifera* (*U.P*) 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/day 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 opposit direction by using methanol (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/day. Tables (1 and 2) showed that *U. P*. has a high antioxidant activity although it was used in low concentration ,this indicats 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 phytochemicl investigation of the plants showed the presence of flavonoids, coumarins, some sterols, alkaloids and hydrocarbons by methanolic extracts meanwhile sterols, fatty acids and terpenes were presented in petroleum ether extract. Flavonoids, phenolic acids and some terpenoids 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 supple-ment or in pharmaceutical industry. In addition, free radicals have been demons-trated 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-Larrca, 1997.).

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دراسة بعض العوامل المضادة للأكسدة على كبد الفئران المعالجة بمستخلص نبات الحريق

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الحريق نبات شعبي وجد له تأثير مضاد للأكسدة في الفئران حيث درس تأثير مستخلص النبات الميثيلى والايثير البترولي للأوراق ,الجذور والبذور بتركيزين مختلفين على كمية البروتين ,الجلوتاثيون والليبيدات المؤكسدة بالإضافة إلى بعض الإنزيمات المضادة للأكسدة مثل جلوتاثيون ترانسفيراز ,الريداكتيز ,البيروكسيداز والإنزيم الذي يحول الشق الهيدروكسيلى إلى بيروكسيد. وقد أثبتت الدراسة أن مستخلص الميثانول المائي أكثر تأثير على القياسات المختلفة من مستخلص الايثير البترولى.وبذلك يمكن استخدام هذا النبات في الصناعات الغذائية والدوائية.