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Short communication

In vivo antioxidant potential of *Teucrium polium*, as compared to α-tocopherol

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The present study was undertaken to explore antioxidant potential of *Teucrium polium* (*Lamiaceae*) *in vivo*. Antioxidant activity was measured by three tests including inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, total antioxidant power (TAP), and thiobarbituric acid reactive substances (TBARS) in serum. Rats received dry extract of *T. polium* in 80% ethanol by intragastric intubation at doses of 50, 100 and 200 mg kg^{-1} daily for 14 days. Treatment of rats with *T. polium* extract showed significant antioxidant activity in the DPPH test as compared to the control. *T. polium* extract at doses of 50 and 100 mg kg^{-1} significantly increased rats TAP and decreased TBARS compared to the control. Administration of *T. polium* at a dose of 200 mg kg^{-1} per day did not significantly alter serum TAP and TBARS. Antioxidant activities of *T. polium* at a doses of 50 and 100 mg kg^{-1} were in all experiments comparable to that of α-tocopherol (10 mg kg^{-1}).

Keywords: *Teucrium polium* L. (*Lamiaceae*), antioxidant, DPPH, TAP, TBARS, α-tocopherol

Medicinal plants are considered to be an important source of antioxidant compounds and the therapeutic benefit of many medicinal plants is often attributed to their antioxidant properties (1, 2). *Teucrium polium* (*Lamiaceae*) is a wild-growing flowering plant, found abundantly in South-Western Asia, Europe and North Africa. *T. polium* is well known for its diuretic, antipyretic, diaphoretic, antispasmodic, tonic, anti-inflammatory, antihypertensive, anorexic, analgesic (3, 4), antibacterial (5) and antidiabetic effects (6). Recently, it has been reported that the extract of *T. polium* reduces NADPH-initiated lipid peroxidation in rat liver microsomes *in vitro* (7). Plants belonging to the genus *Teucrium* have been shown to contain different classes of compounds such as fatty acid esters (8), diterpenes (9), monoterpenes (10), sesquiterpenes (11), flavonoids and polyphenolics (12, 13). Flavonoids that have been isolated from *T. polium* species include cirsimaritin, cirsilol, cirsilineol, 5-hydroxy-6,7,3′,4′-tetramethoxyflavone, salvigenin, apigenin 5-galloylglucoside, apigenin-7-glucoside, vicenin-2- and luteolin-7-glucoside (6, 12, 14). The antioxidant potential of cirsimaritin and apigenin-7-glucoside has been indicated (15). Vicenin

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and luteolin-7-glucoside have shown the strongest antioxidant activity in *in vitro* tests (16). Polyphenolic compounds have also shown strong antioxidant activity (14). The positive effects of these antioxidant components come from their ability to inhibit lipid peroxidation and chelate redox-active metals (17). There is evidence that flavonoids have anti-phosphodiesterase activity and could thus elevate intracellular levels of cyclic nucleotides (18). Recent studies indicate that both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) can diminish oxidative stress in many biological systems and diseases *in vivo* (19, 20).

Because of the effect of free radicals on all cellular macromolecules, which result in various diseases, it seemed interesting to study the antioxidant potential of *T. polium in vivo*.

EXPERIMENTAL

Plant material and total extract

Aerial parts of *T. polium* were collected during the flowering period in the region of Kerman borders (East Iran). Samples of the plant were identified by a botanist from the Division of Pharmacognosy, Tehran University of Medical Sciences (TUMS), Iran.

The plant material was air dried, powdered and extracted by the percolation method using ethanol (80%). The solvent was removed under vacuum in a rotary evaporator until dryness (14%, *m/m*, of dry plant).

Chemicals

All chemicals were of highest purity (≥ 99.0%). Sodium acetate, 2,4,6-tripyridyl-s-triazine (TPTZ), 2-thiobarbituric acid (TBA), 1,1,3,3-tetramethoxypropan (MDA), trichloroacetic acid (TCA), glacial acetic acid, 1,1-diphenyl-2-picryl hydrazyl (DPPH), $FeCl_3 \times 6\ H_2O$, HCl, and *n*-butyl alcohol were purchased from Merck Iran. α-Tocopherol (Trolox) was purchased from ACROS organics, Belgium.

Animals and treatment

Experiments were performed on adult male Wistar rats from the Pasteur Institute of Tehran (Iran) weighing 180–200 g. They were kept under standardized conditions (temperature 21–24 °C and a light/dark cycle of 12 hours/12 hours) and fed a normal laboratory diet. After 1 week of acclimatization, rats were divided into one control and four experimental groups with 6 animals in each group. The study protocol was approved by the Pharmaceutical Sciences Research Center (PSRC)/TUMS Ethics Committee.

The extract was dissolved in normal saline to provide a 20 mg mL^{-1} solution. Animals from group 1 to 3 received doses expressed on the basis of mg dry extract per kg body mass, namely 50, 100, and 200 mg kg^{-1} per day of the extract by intragastric intubation for 14 days. Group 4 received α-tocopherol (10 mg kg^{-1} per day) dissolved in saline by intragastric intubation as a reference antioxidant for comparison. The fifth group of animals was treated as control and received only saline.

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Blood collection

About 4 mL of blood was collected through direct heart puncture from anesthetized rats. Intraperitoneal administration of pentobarbital (60 mg kg^{-1}) was used to induce anesthesia in rats. The blood was centrifuged at 2000 × *g* for 10 minutes to separate serum. The serum was kept at –20 °C for subsequent determination of lipid peroxidation and antioxidant status.

Lipid peroxidation assay

Thiobarbituric Acid Reactive Substances (TBARS) assay is the method of choice for screening and monitoring lipid peroxidation, a major indicator of oxidative stress. To precipitate the serum proteins, 2.5 mL of TCA 20% (*m/V*) was added into 0.5 mL of the sample, which was then centrifuged at 1500 × *g* for 10 min. Then 2.5 mL of sulfuric acid (0.05 m L^{-1}) and 2 mL TBA (0.2%) was added to the sediment, shaken, and incubated for 30 min in a boiling water bath. Then, 4 mL *n*-butanol was added, and the solution was centrifuged, cooled and the supernatant absorption was recorded at 532 nm using a UV-Visible spectrophotometer (Shimadzu, Japan). The calibration curve was obtained using different concentrations of 1,1,3,3-tetramethoxypropane as standard to determine the concentration of TBA-MDA adducts in samples (21).

Total antioxidant power (TAP) assay

The total antioxidant capacity of serum was determined by measuring its ability to reduce Fe^{3+} to Fe^{2+} by the FRAP (Ferric Reducing Ability of Plasma) test. The FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe(II)-tripyridyltriazine compound from Fe(III) by the action of electron donating antioxidants. The FRAP reagent consists of 300 mmol L^{-1} acetate buffer pH = 3.6, 10 mmol L^{-1} TPTZ in 40 mmol L^{-1} HCl and 20 mmol L^{-1} $FeCl_3 \times 6\,H_2O$ in the ratio of 10:1:1. Briefly, 10 µL of serum was added to 300 µL freshly prepared and prewarmed (37 °C) FRAP reagent in a test tube and incubated at 37 °C for 10 min. The absorbance of the blue colored complex was read against a reagent blank (300 µL FRAP reagent + 10 µL distilled water) at 593 nm. Standard solutions of Fe^{2+} in the range of 100 to 1000 mmol L^{-1} were prepared from ferrous sulphate ($FeSO_4 \times 7\,H_2O$) in water. The data was expressed as mmol ferric ions reduced to ferrous form per liter (FRAP value) (22).

DPPH radical scavenging activity

In this test, serum ability to inhibit DPPH radical was measured (23). DPPH is one of the few stable organic nitrogen radicals and has a maximum of absorption at 517 nm. 20 µL of rat's serum was added to 3 mL of DPPH solution (0.1 mmol L^{-1} in ethanol) and the reaction mixture was shaken vigorously. After incubation at room temperature for 10 min, the absorbance of this solution was determined at 517 nm DPPH solutions without serum and with α-tocopherol were used as the control and reference, respectively.

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Determination of LD_{50}

In order to determine the acute toxicity (LD_{50}) of *T. polium*, doses of 10, 100, 1000, and 2000 mg kg^{-1} of the day extract were administrated to rats via intragastric tube. The animals were observed for 48 h and mortality was recorded at the end of this period (24).

Statistical analysis

The values are reported as mean ± SEM. One-way ANOVA and Tukey posthoc multicomparison tests were used for data analysis.

RESULTS AND DISCUSSION

The acute toxicity test (LD_{50}) demonstrated that *T. polium* extract is not lethal up to a dose of 2000 mg kg^{-1}.

T. polium extracts in all doses (mg kg^{-1}) used significantly ($p < 0.05$) increased the serum DPPH scavenging potential when compared to the control as follows: 50 (786%), 100 (907%), 200 (779%). This value for α-tocopherol (10 mg kg^{-1}) compared to the control was 667% ($p < 0.05$) (Fig. 1a). *T. polium* extract in the same doses (mg kg^{-1}), significantly ($p < 0.005$) increased the serum TAP when compared to the control as follows: 50 (23.6%), 100 (37.5%) (Fig. 1b) and decreased the serum TBARS when compared to the control as follows: 50 (24.7%), 100 (31.8%) (Fig. 1c). This value for α-tocopherol (10 mg kg^{-1}) compared to the control in the TAP assay was 16.8% ($p < 0.05$) and in the TBARS assay 29.5% ($p < 0.05$). *T. polium* at a dose of 200 mg kg^{-1} per day did not significantly alter the serum TAP and TBARS.

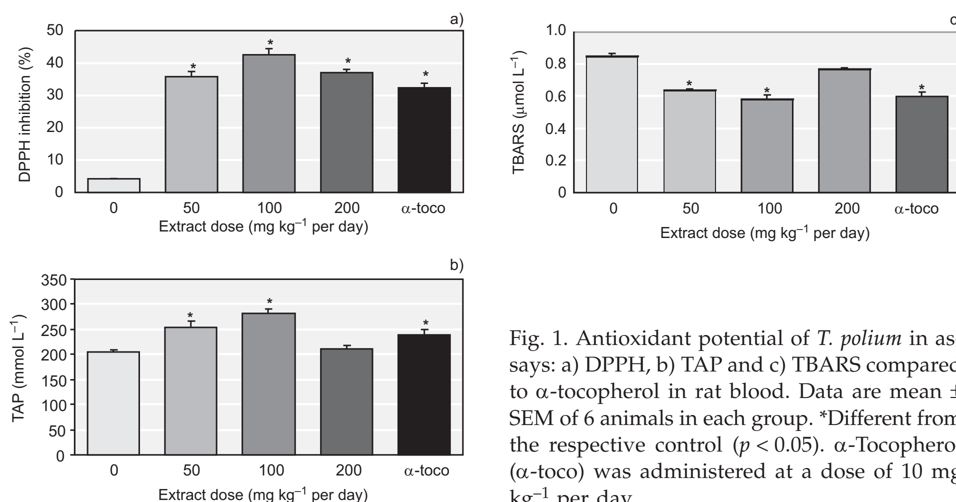

Fig. 1. Antioxidant potential of *T. polium* in assays: a) DPPH, b) TAP and c) TBARS compared to α-tocopherol in rat blood. Data are mean ± SEM of 6 animals in each group. *Different from the respective control ($p < 0.05$). α-Tocopherol (α-toco) was administered at a dose of 10 mg kg^{-1} per day.

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Data obtained by DPPH, FRAP, and TBARS assays indicate that *T. polium* effectively inhibits oxidative stress *in vivo*. When tested by FRAP and TBARS, *T. polium* extract at a dose of 200 mg kg^{-1} per day exhibited no significant antioxidant activity in comparison to the control. It is proposed that the DPPH test is more sensitive than FRAP and TBARS tests for examination of the antioxidant capacity of *T. polium*.

Results of the present *in vivo* study are supported by the work of Panovska and Kulevanova (7) indicating that diethyl ether, ethyl acetate and *n*-butanol extracts of *T. species* inhibit lipid peroxidation *in vitro*. In addition, they indicated that the inhibitory effect of the extracts was greater than that of reference substances such as luteolin, and similar to that of thymol and butylated hydroxyl toluene. Regarding the presence of flavonoids and polyphenolic compounds such as cirsimaritin, apigenin-7-glucoside, vicenin, and luteolin-7-glucoside in the *T. polium* extract (15, 16), the obtained *in vivo* results are not surprising. As mentioned earlier, *T. polium* has been recognized in folk medicine in the treatment of diabetes (6). Regarding the role of oxidative stress in the pathogenesis of diabetes (26), the benefit of *T. polium* in diabetes seems reasonable.

CONCLUSIONS

This preliminary study indicates the interesting antioxidative stress potential of *T. polium in vivo* that is comparable to that of α-tocopherol. Further studies are needed to elucidate whether *T. polium* could be useful in the management of human diseases resulting from oxidative stress.

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REFERENCES

1. Z. Zhang, Q. Chang, M. Zhu, Y. Huang, W. K. K. Ho and Z. Y. Chen, Characterization of antioxidants present in hawthorn fruits, *J. Nut. Biochem.* **12** (2001) 144–152.
2. C. Rice-Evans, Flavonoids and isoflavones: absorption, metabolism and bioactivity, *Free Radical Biol. Med.* **36** (2004) 827–828.
3. M. S. Suleiman, A. S. Abdul-Ghani, S. Al-Khali and R. Amin, Effect of Teucrium polium boiled leaf extract on intestinal motility and blood pressure, *J. Ethnopharmacol.* **22** (1998) 111–116.
4. M. Tariq, A. M. Ageel, M. A. Al-Yahia, J. S. Mossa and M. S. Al-Said, Anti-inflammatory activity of *Teucrium polium*, *Int. J. Tissue React.* **11** (1989) 185–188.
5. S. Mansouri, Inhibition of *Staphylococcus aureus* mediated by extracts of Iranian plants, *Pharmac. Biol.* **37** (1999) 375–377.
6. M. A. Esmaeili and R. Yazdanparast, Hypoglycaemic effect of *Teucrium polium*: studies with rat pancreatic islets, *J. Ethnopharmacol.* **95** (2004) 27–30.
7. T. K. Panovska and S. Kulevanova, Effect of some *Teucrium* species (*Lamiaceae*) on lipid peroxidation in rat liver microsomes, *Fresenius Environ. Bull.* **14** (2005) 957–959.

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8. G. Fonta, G. Savona and B. Redriguez, Usual 6'-fatty acid esters of (24S-24-ethyl-cholesta-5,25-dien-3β-yl-β-D-Glucopyranoside) from *Teucrium polium*, *J. Ethnopharmacol.* **24** (1999) 93–99.
9. D. A. Mini, P. Zhang, X. Zhao, S. Wang Chong and Q. Zheno, A neo clerodane diterpene from *Teucrium japonicum*, *Phytochemistry* **30** (1991) 4175–4177.
10. S. Cozzani, A. Muselli, J. M. Desjobert, A. F. Bernardini and F. Tomi, Chemical composition of essential oil of *Teucrium polium* subsp. *capitatum* (L.) from Corsica, *J. Essent. Oil Res.* **10** (2005) 113–115.
11. P. Uma Devi, A. Ganasoundari, B. Vrinda, K. K. Srinivasan and M. K. Unnikrishnan, Radiation protection by the ocimum flavonoids orientin and vicenin: mechanisms of action, *Radiat. Res.* **154** (2000) 455–460.
12. J. B. Harborne, B. Tomas, G. A. Williams and M. I. Gil, A chemotoxonomic study of flavonoids from European *Teucrium* species, *Phytochemistry* **25** (1986) 2811–2816.
13. A. M. Rizk, F. M. Hammouda, H. Rimpler and A. Kamel, Iridoids and flavonoids of *Teucrium polium* herb, *Planta Medica* **2** (1986) 87–88.
14. T. Kadifkova Panovska, S. Kulevanova and M. Stefova, *In vitr*o antioxidant activity of some *Teucrium* species (*Lamiaceae*), *Acta Pharm.* **55** (2005) 207–214.
15. M. E. Cuvelier, C. Berset and H. Richard, Antioxidant constituents in sage (*Salvia officinalis*), *J. Agric. Food Chem.* **42** (1994) 665–669.
16. K. Ulubelen, G. Topcu and U. Kaya, Steroidal compounds from *Teucrium chamaedrys* subsp. chamaedrys, *Phytochemistry* **36** (1994) 171–173.
17. L. Le Marchand, Cancer preventive effects of flavonoids – a review, *Biomed. Pharmacother.* **56** (2002) 296–301.
18. M. Abdollahi, T. S. Chan, V. Subrahmanyam and P. J. O'Brien, Effects of phosphodiesterase 3,4,5 inhibitors on hepatocyte cAMP levels, glycogenolysis, gluconeogenesis and susceptibility to a mitochondrial toxin, *Mol. Cell. Biochem.* **252** (2003) 205–211.
19. E. Milani, S. Nikfar, R. Khorasani, M. J. Zamani and M. Abdollahi, Reduction of diabetes-induced oxidative stress by phosphodiesterase inhibitors in rats, *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **140** (2005) 251–255.
20. R. Aghababaeian, M. Ghazi-Khansari, K. Abdi, F. Taghadosinejad and M. Abdollahi, Protective effects of sildenafil and dipyridamol from lead-induced lipid peroxidation in perfused rat liver, *Int. J. Pharmacol.* **1** (2005) 157–160.
21. K. Satho, Serum lipid peroxidation in cerebrovascular disorders determined by a new colorimetric method, *Clin. Chim. Acta* **90** (1978) 37–43.
22. I. F. Benzie and J. J. Strain, Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay, *Anal. Biochem.* **239** (1996) 70–76.
23. T. Yokozawa, C. P. Chen, E. Dong, T. Tanaka, G. I. Nonaka and I. Nishioka, Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2 picrylhydrazyl radical, *Biochem. Pharmacol.* **56** (1998) 213–222.
24. A. W. Hayes, *Principles and Methods of Toxicology*, 2nd ed., Raven Press, New York 1988, pp. 169–221.
25. A. Ceriello, New insights on oxidative stress and diabetic complications may lead to a »causal« antioxidant therapy, *Diabetes Care* **26** (2003) 1589–1596.

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S A Ž E T A K

In vivo antioksidativni potencijal biljke *Teucrium polium* u usporedbi s α-tokoferolom

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U okviru ovih istraživanja ispitan je antioksidativni potencijal biljke *Teucrium polium (Lamiaceae) in vivo.* Antioksidativni učinak mjeren je pomoću tri testa koji uključuju inhibiciju 1,1-difenil-2-pikrilhidrazil (DPPH) radikala, ukupnu antioksidativnu snagu (TAP) i reaktivne supstancije tiobarbiturne kiseline (TBARS) u serumu. Štakorima je davan suhi ekstrakt *T. polium* u 80%-tnom etanolu intragastričnom intubacijom u dozama od 50, 100 i 200 mg kg^{-1} dnevno tijekom 14 dana. Pokusi su pokazali značajno antioksidativno djelovanje *T. polium* DPPH testom u usporedbi s kontrolom. *T. polium* je u dozama od 50 i 100 mg kg^{-1} značajno povisio TAP i snizio TBARS u usporedbi s kontrolom. Primjena ekstrakta *T. polium* u dozi od 200 mg kg^{-1} dnevno nije značajno mijenjala serumske TAP i TBARS vrijednosti. Antioksidativni učinak *T. polium* u dozama 50 i 100 mg kg^{-1} bio je u svim eksperimentima sličan učincima α-tokoferola (10 mg kg^{-1}).

Preliminarna ispitivanja ukazuju na antistresni učinak *T. polium* koji je usporediv antioksidativnom učinku. Međutim, potrebna su daljnja ispitivanja da se rasvijetli bi li *T. polium* mogla biti korisna u uklanjanju posljedica oksidativnog stresa.

Ključne riječi: *Teucrium polium* L. *(Lamiaceae)*, antioksidans, DPPH, TAP, TBARS, α-tokoferol

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