International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 3 (6): 510-514, 2007
ISSN 1811-7775
© 2007 Asian Network for Scientific Information

On the Anti Oxidative Stress Potential of *Zataria multiflora* Boiss (*Avishan shirazi*) in Rats

[1,2]Mona Babaie, [2]Narguess Yasa, [1]Azadeh Mohammadirad, [1]Reza Khorasani and [1]Mohammad Abdollahi
[1]Laboratory of Toxicology, Faculty of Pharmacy and Pharmaceutical Sciences Research Centre,
[2]Laboratory of Pharmacognosy, Faculty of Pharmacy and Medicinal Plants Research Center,
Tehran University of Medical Sciences, Tehran, Iran

Abstract: The present study was undertaken to explore the antioxidants effects of *Zataria multiflora* Boiss in rats. Antioxidant activity was measured by inhibition of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, Total Antioxidant Power (TAP) and Thiobarbituric Acid Reactive Substances (TBARS) in serum of treated rats. Rats received methanolic extract of *Z. multiflora* by intragastric intubation at doses of 50, 100 and 200 mg kg^{-1} daily for 14 consecutive days. The acute toxicity test (LD_{50}) demonstrated that *Z. multiflora* is not lethal up to a dose of 2000 mg kg^{-1} after oral administration. Treatment of rats with *Z. multiflora* extract showed significant antioxidant activity in the DPPH test as compared to the control. *Z. multiflora* at doses of 50 and 100 mg kg^{-1} significantly increased TAP and decreased TBARS as compared to the control. Administration of *Z. multiflora* at a dose of 200 mg kg^{-1} per day did not significantly alter serum DPPH, TAP and TBARS. Antioxidant activities of *Z. multiflora* at doses of 50 and 100 mg kg^{-1} were in all experiments comparable to that of α-tocopherol. Further studies are needed to elucidate whether *Z. multiflora* as herbal medicine could be useful in the management of human diseases resulting from oxidative stress.

Key words: *Z. multiflora*, antioxidant, DPPH, TAP, TBARS, α-tocopherol

INTRODUCTION

Oxidative stress results from an imbalance between the generation of oxygen derived radicals and the organism's antioxidant potential and thus plays important role in pathogenesis of many chronic diseases. Antioxidants are generally believed to protect body against oxidative stress by several mechanisms. These mechanisms include enzymatic degradation of free radicals, binding metals which stimulate the production of free radicals and scavenging free radicals (Abdollahi *et al*., 2004). Antioxidants may prevent the development of many chronic diseases associated with oxidative stress like cancer, heart failure, diabetes, Alzheimer and many other harmful diseases (Aro, 2003; Polidori, 2003; Ferrari *et al*., 2004).

Medicinal plants are considered as an important source of antioxidant compounds. Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones.

The family of Labiatae are generally known for their various effects such as analgesic and anti-inflammatory activity (Hernandez-Perez *et al*., 1995), antioxidant (Cuppett and Hall, 1998), hepatoprotective (Wasser *et al*., 1998) and hypoglycemic effects (Hosseinzadeh *et al*., 1998). *Z. multiflora* is a plant from that family that is distributed only in Iran, Pakistan and Afghanistan. It is greatly used for medicinal and condimental purposes in these countries. This plant with the vernacular name of *Avishan shirazi* in Iran has several traditional uses such as antiseptic, anesthetic and antispasmodic (Zargari, 1990). Our recent study confirmed its anti-colitis effect in experimental animals (Ashtaral-Nakhai *et al*., 2007). Our recent study provided evidence of the benefit effects of some plants in rats (Hasani *et al*., 2007; Shahriari *et al*., 2006) by reduction of blood Lipid Peroxidation (LP) and increased blood Total Antioxidant Power (TAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Regarding these reports, we hypothesized that *Z. multiflora* may have anti oxidative stress potential in rat.

MATERIALS AND METHODS

Plants material: Samples of *Z. multiflora* were collected from Shiraz, Iran, on 20 may 2006. The leaves of the plant was dried in shadow and stored in the Department of Botany of the Research Institute of Forests and

Corresponding Author: Mohammad Abdollahi, Faculty of Pharmacy and Pharmaceutical Sciences Research Centre, Tehran University of Medical Sciences, Tehran, Iran Tel/Fax: +98 216 6959104

Ranglands (TARI), Tehran. A voucher specimen (No. 58416) has been deposited at the Herbarium of TARI.

Preparation of total extract: Amount of 86.4 kg of plant powder was wet with a solvent (methanol) in a closed plastic container; then the wet powder put in a percolator and was macerated in 10 L methanol (100% v/v) for 24 h and subsequently, the solution was filtered and concentrated in a percolator by 100 drops per min. This procedure was repeated twice and three times with 10 L methanol (100% v/v), respectively. The extract was then concentrated under reduced pressure and appropriate temperature and the solvent was distilled in vacuum and finally 1.5 kg solid solvent was produced.

Materials: 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-hydrazyl (DPPH), $FeCl_3 . 6 H_2O$, HCl and n-butyl alcohol were purchased from Merck (Tehran). α-Tocopherol (Torolox) was purchased from ACROS organics, Belgium.

Animals and treatment: Experiments were performed on adult male Wistar rats from the *Pasteur* Institute of Tehran weighing 180-200 g. They were kept under standardized conditions (temperature 21-24°C and a light/dark cycle of 12/12 h 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.

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 min to separate serum. The serum was kept at -20°C for subsequent determination of biochemical parameters.

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 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 sample (Satho, 1978).

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 antioxidant. 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} $FeCl_3 . 6H_2O$ 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 ($FeSO_4 . 7H_2O$) in water. The data was expressed as mmol ferric ions reduced to ferrous form per litter (FRAP value) (Benzie and Strain, 1996).

DPPH radical scavenging activity: In this test, serum ability to inhibit DPPH radical was measured (Yokozawa *et al*., 1998). DPPH is one of the few stable organic nitrogen radicals and has a maximum of absorption at 517 nm 20 μL of 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.

Determination of LD_{50}: In order to determine the acute toxicity (LD_{50}) of *Z. multiflora*, 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 (Hayes, 1988).

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 *Z. multiflora* extract is not lethal up to a dose of 2000 mg kg^{-1} and no sign of toxicity was observed and thus is considered non-toxic. *Z. multiflora* extract in doses of 50 and 100 mg kg^{-1} ($p<0.05$) increased the serum DPPH scavenging potential when compared to the control as follows: 50 (76.3%), 100 (135%). This value for α-tocopherol (10 mg kg^{-1}) as compared to the control was 116% ($p<0.05$) (Fig. 1). *Z. multiflora* extract in the same doses (mg kg^{-1}), significantly ($p<0.05$) increased the serum TAP when compared to the control as follows: 50 (63%) and 100 (74.5%) (Fig. 2). They also decreased the serum TBARS when compared to the control as follows: 50 (63%) and 100 (60.7%) (Fig. 3). This values for α-tocopherol (10 mg kg^{-1}) in the TAP and TBARS assays were 67.7% ($p<0.05$) and 69.9% ($p<0.05$), respectively. *Z. multiflora* at dose of 200 mg kg^{-1} per day did not significantly alter the serum DPPH, TAP and TBARS.

Data obtained by DPPH, FRAP and TBARS assays indicate that *Z. multiflora* effectively inhibits oxidative stress *in vivo*. The composition of the essential oil of *Z. multiflora* was studied by GLC, Column Chromatography (CC), NMR and GLC/MS (Ebrahimzadeh *et al*., 2003; Mohagheghzadeh *et al*., 2000; Shaiq *et al*., 1999; Shafiee and Javidnia, 1997). Regarding above studies, Zatarinal, β-sitosterol, stigmasterol, oleanolic acid, betulinic acid, hexadecanoic, luteolin, α-tocophorolquinone and Rosmarinic Acid (RA) were reported as the composition of *Z. multiflora* essential oil. On the other hand, phytochemical screening of ethanolic extract of the plant supported the presence of monoterpen phenolic compounds in *Z. multiflora* (Ali *et al*., 2000; Ramesh *et al*., 1998; Martinez-Vazquez *et al*., 1996), mainly carvacrol, p-cymene, thymol, linalool and γ-terpinene (Mohagheghzadeh *et al*., 2000). RA as a flavonoid from *Z. multiflora* extract is has significant antioxidant and chelating properties. This positive effect can result in reduction of free radical-induced damages in the body. In supporting this idea, there is evidence that flavonoids have anti phosphodiesterase activity and thus could elevate intracellular levels of cyclic nucleotides

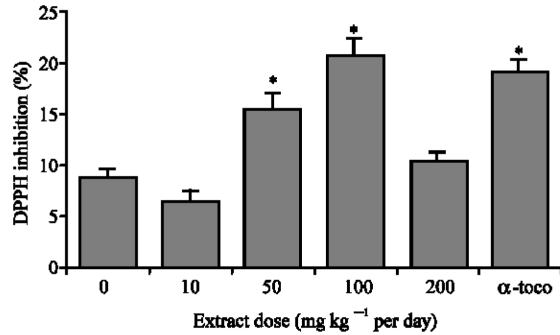

Fig. 1: Antioxidant potential of *Z. multiflora* in DPPH assay compared to α-tocopherol in rat blood. Data are mean±SEM of 6 animals in each group. *Different from the respective control ($p<0.05$). α-Tocopherol (α-toco) was administered at a dose of 10 mg kg^{-1} per day

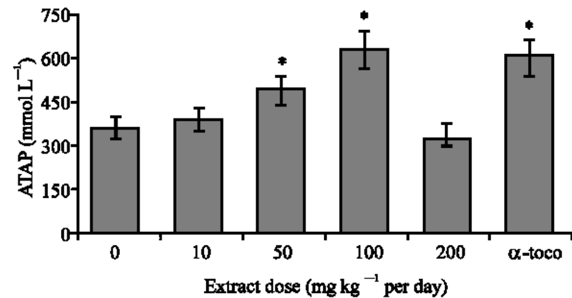

Fig. 2: Antioxidant potential of *Z. multiflora* in TAP assay compared to α-tocopherol in rat blood. Data are mean±SEM of 6 animals in each group. *Different from the respective control ($p<0.05$). α-Tocopherol (α-toco) was administered at a dose of 10 mg kg^{-1} per day

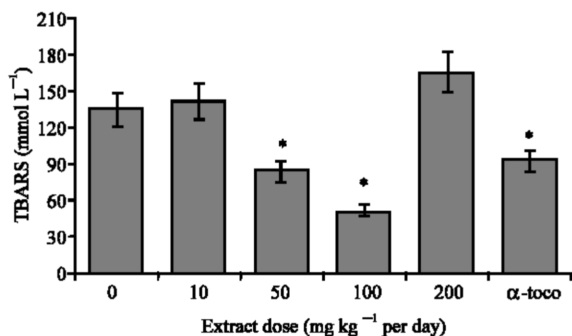

Fig. 3: Antioxidant potential of *Z. multiflora* in TBARS assay compared to α-tocopherol in rat blood. Data are mean±SEM of 6 animals in each group. *Different from the respective control ($p<0.05$). α-Tocopherol (α-toco) was administered at a dose of 10 mg kg^{-1} per day

(Abdollahi *et al*., 2003a). Recent studies well indicate that both cAMP and cGMP can diminish oxidative stress in many biological systems and diseases (Aghababaeian *et al*., 2005; Milani *et al*., 2005; Radfar *et al*., 2005; Abdollahi *et al*., 2003b; Abdollahi *et al*., 2003c). Therefore, the beneficial effects of *Z. multiflora* in oxidative stress mainly back to its strong antioxidant potential.

Results indicated that *Z. multiflora* does not act dose-dependently and dose of 200 mg kg^{-1} was ineffective. In explanation, it has to be mentioned that the extract has several compounds that some of them at higher doses may produce an unknown condition leading to hiding of antioxidant effects. This will be elucidated by examination of each components of this extract separately.

This preliminary study indicates the interesting antioxidative stress potential of *Z. multiflora in vivo* that is comparable to that of α-tocopherol and further supports our recent findings about anti colitis effects of *Z. multiflora* in mice. Further studies are needed to elucidate whether *Z. multiflira* could be useful in the management of human diseases resulting from oxidative stress.

ACKNOWLEDGMENT

This study was financially supported by a grant from Pharmaceutical Sciences Research Center, TUMS.

REFERENCES

Abdollahi, M., T.S. Chan, V. Subrahmanyam and P.J. O'Brien, 2003a. Effects of phosphodiesterase 3,4,5 inhibitors on hepatocyte cAMP levels, glycogenolysis, gluconeogenesis and susceptibility to a mitochondrial toxin. Mol. Cell. Biochem., 252: 205-211.

Abdollahi, M., A. Bahreini-Moghadam, B. Emami, F. Fooladian and K. Zafari, 2003b. Increasing intracellular cAMP and cGMP inhibits cadmium-induced oxidative stress in rat submandibular saliva. Comp. Biochem. Physiol. C. Toxicol. Pharmacol., 135: 331-336.

Abdollahi, M., F. Fooladian, B. Emami, K. Zafari and A. Bahreini-Moghadam, 2003c. Protection by sildenafil and theophylline of lead acetate-induced oxidative stress in rat submandibular gland and saliva. Hum. Exp. Toxicol., 22: 587-592.

Abdollahi, M., A. Ranjbar, S. Shadnia, S. Nikfar and A. Rezaie, 2004. Pesticides and oxidative stress: A review. Med. Sci. Monit., 10: RA141-14.

Aghababaeian, R., M. Ghazi-Khansari, K. Abdi, F. Taghadosinejad and M. Abdollahi, 2005. Protective effects of sildenafil and dipyridamol from lead-induced lipid peroxidation in perfused rat liver. Int. J. Pharmacol., 1: 157-160.

Ali M.S., M. Saleem, Z. Ali and V.U. Ahmad, 2000. Chemistry of *Zataria multiflora* (Lamiaceae). Phytochemistry, 55 (8): 933-936.

Aro, A., 2003. Antioxidant supplementation and risk of chronic disease. Forum. Nutr., 56: 361-363.

Ashtaral-Nakhai, L., A. Mohammadirad, N. Yasa, B. Minaie, S. Nikfar, G. Ghazanfari, M.J. Zamani, G. Dehghan, H. Jamshidi, V. Shetab-Boushehri, R. Khorasani and M. Abdollahi, 2007. Benefits of *Zataria multiflora* Boiss in experimental model of mouse inflammatory bowel disease. Evid. Based Complement. Alt. Med., 4 (1): 43-50.

Benzie, I.F. and J.J. Strain, 1996. Ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal. Biochem., 239: 70-76.

Cuppett, S.L. and C.A. Hall, 1998. 3rd Antioxidant activity of the Labiatae. Adv. Food. Nutr. Res., 42: 245-271.

Ebrahimzadeh, H., Y. Yamini, F. Sefidkon, M. Chaloosi and S.M. Pourmortazavi, 2003. Chemical composition of the essential oil and supercritical CO_2 extracts of *Zataria multiflora* Boiss. Food Chem., 83: 357-361.

Ferrari, R., G. Guardigli, D. Mele, G.F. Percoco, C. Ceconi and S. Curello, 2004. Oxidative stress during myocardial ischaemia and heart failure. Curr. Pharm. Des., 10: 1699-1711.

Hasani, P., N. Yasa, S. Vosough-Ghanbari, A. Mohammadirad, G.H. Dehghan and M. Abdollahi, 2007. *In vivo* antioxidant potential of *Teucrium polium*, as compared to α-tocopherol. Acta Pharm., 57: 123-129.

Hayes, A.W., 1988. Principles and Methods of Toxicology. 2nd Edn. Raven Press, New York, pp: 169-221.

Hernandez-Perez, M., R.M. Rabanal, M.C. de, la, Torre and B. Rodriguez, 1995. Analgesic, anti-inflammatory, antipyretic and haematological effects of aethiopinone, an o-naphthoquinone diterpenoid from *Salvia aethiopis* roots and two hemisynthetic derivatives. Planta Med., 61: 505-509.

Hosseinzadeh, H., H.M.H. Khodaparast and H. Shokohizadeh, 1998. Antihyperglycaemic effect of *Saliva leriifolia* Benth leaf and seed extract in mice. Iranian J. Med. Sci., 23: 78-80.

Martinez-Vazquez, M., T.O. Ramirez, Apan, H. Aguilar and R. Bye, 1996. Analgesic and antipyretic activities of an aqueous extract and of the flavone linarin of *Buddleia cordata*. Planta. Med., 62: 137-140.

Milani, E., S. Nikfar, R. Khorasani, M.J. Zamani and M. Abdollahi, 2005. Reduction of diabetes-induced oxidative stress by phosphodiesterase inhibitors in rats. Comp. Biochem. Physiol. C. Toxicol. Pharmacol., 140: 251-255.

Mohagheghzadeh, A., M. Shams-Ardakani and A. Ghannadi, 2000. Volatile constituents of callus and flower-bearing tops of *Zataria multiflora* Boiss (Lamiaceae). Flav Frag J., 15: 373-376.

Polidori, M.C., 2003. Antioxidant micronutrients in the prevention of age-related diseases. J. Postgrad Med., 49: 229-235.

Radfar, M., B. Larijani, M. Hadjibabaie, B. Rajabipour, A. Mojtahedi and M. Abdollahi, 2005. Effects of pentoxifylline on oxidative stress and levels of EGF and NO in blood of diabetic type-2 patients. A randomized, double-blind placebo-controlled clinical trial. Biomed. Pharmacoth., 59: 302-306.

Ramesh, M., Y.N. Rao, A.V. Rao, M.C. Prabhakar, C.S. Rao, N. Muralidhar and B.M. Reddy, 1998. Antinociceptive and anti-inflammatory activity of a flavonoid isolated from *Caralluma attenuata*. J. Ethnopharmacol., 62: 63-66.

Satho, K., 1978. Serum lipid peroxidation in cerebrovascular disorders determined by a new colorimetric method. Clin. Chim. Acta, 90: 37-43.

Shafiee, A. and K. Javidnia, 1997. Composition of essential oil of *Zataria multiflora*. Plant. Media., 63: 371-372.

Shahriari, S., N. Yasa, A. Mohammadirad, R. Khorasani and M. Abdollahi, 2006. *In vivo* antioxidant potentials of rosa damascene petal extract from Guilan, Iran, comparable to α-tocopherol. Int. J. Pharmacol., 2 (6): 646-649.

Shaiq, Ali, M., M. Jahangir, M. Saleem and V. Uddin, Ahmad, 1999. Chemical constituents of *Pulicaria gnaphalodes*. Nat. Prod. Sci., 5: 134-137.

Wasser, S., J.M. Ho, H.K. Ang and C.E. Tan, 1998. Salvia miltiorrhiza reduces experimentally-induced hepatic fibrosis in rats. J. Hepatol., 29: 760-771.

Yokozawa, T., C.P. Chen, E. Dong, T. Tanaka, G.I. Nonaka and I. Nishioka, 1998. Study on the inhibitory effect of tannins and flavonoids against the 1,1-diphenyl-2 picrylhydrazyl radical. Biochem. Pharmacol., 56: 213-222.

Zargari, A., 1990. Medicinal Plants. 4th Edn. Tehran University Press, Tehran, pp: 1-57.