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

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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 (LD₅₀) 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 
one.

The family of Labiatae are generally known for their 
various effects such as analgesic and anti-inflammatory 
activity (Hernandez-Perez et al., 1995), antioxidant 
(Cuppelt 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 (Ashtaran-Nakhi 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
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 Fe²⁺ to Fe³⁺ 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)-tripryridyltriazine 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 FeCl₃·6H₂O 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 Fe²⁺ in the range of 100 to 1000 mmol L⁻¹ were prepared from ferrous sulphate (FeSO₄·7H₂O) 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 LD₅₀: In order to determine the acute toxicity (LD₅₀) 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 post hoc 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 \(\alpha\)-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 \(\alpha\)-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 (EbrahimiZadeh et al., 2003; Mohagheghzadeh et al., 2000; Shafiee et al., 1999; Shafiee and Javachia, 1997). Regarding above studies, Zatarial, \(\beta\)-sitosterol, stigmasterol, oleanolic acid, betulinic acid, hexadecanoic, luteolin, \(\alpha\)-tocopherolquinone 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 \(\gamma\)-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 \(\alpha\)-tocopherol in rat blood. Data are mean±SEM of 6 animals in each group. *Different from the respective control (p<0.05). \(\alpha\)-Tocopherol (\(\alpha\)-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 \(\alpha\)-tocopherol in rat blood. Data are mean±SEM of 6 animals in each group. *Different from the respective control (p<0.05). \(\alpha\)-Tocopherol (\(\alpha\)-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 \(\alpha\)-tocopherol in rat blood. Data are mean±SEM of 6 animals in each group. *Different from the respective control (p<0.05). \(\alpha\)-Tocopherol (\(\alpha\)-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. multiflora could be useful in the management of human diseases resulting from oxidative stress.

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REFERENCES


