

Short Report

## The Effect of *Pistacia vera* L. Gum Extract on Oxidative Damage during Experimental Cerebral Ischemia-Reperfusion in Rats

Seyed Mohammad Taghi Mansouri<sup>1</sup>, Bahareh Naghizadeh<sup>1</sup>, Hossein Hosseinzadeh<sup>\*2</sup>

<sup>1</sup>Dept. of Pharmacology, School of Medicine; <sup>2</sup>Pharmaceutical Research Center, Dept. of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

Received 12 December 2004; revised 4 April 2005; accepted 25 April 2005

### ABSTRACT

Oxygen free radicals may be implicated in the pathogenesis of ischemia reperfusion damage. As the antioxidant effects of some species of *Pistacia* have been reported, the protective effects of *Pistacia vera* L. gum extract (0.1-0.5 g/kg) on oxidative damage following cerebral ischemia were studied in rats. Ischemia was induced using four-vessel occlusion model and evaluated using measurement of malondialdehyde (MDA) and antioxidant power in hippocampus. MDA and antioxidant power were assayed with the thiobarbituric acid (TBA) and ferric reducing-antioxidant power (FRAP) tests, respectively. The extract and saline were administered intraperitoneally 10 min subsequent to ischemia. Results have shown that the MDA level increased by 47% and antioxidant power decreased by 117% in control group in comparison with sham-operated animals ( $P < 0.001$ ). Treatment with *P. vera* L. gum extract significantly and in a non-dose-dependent manner reduced brain MDA level by 63% ( $P < 0.001$ ) and increased antioxidant power of brain by 235% ( $P < 0.001$ ) in comparison to the controls. *P. vera* L. gum comprised of saponins, tannins, and flavonoids. According to these results, it is suggested that *P. vera* gum may exhibit neuroprotective effects against ischemia. *Iran. Biomed. J.* 9 (4): 181-185, 2005

**Keywords:** *Pistacia vera*, Oxidative stress, Lipid peroxidation, Global ischemia-reperfusion, Hippocampus

### INTRODUCTION

Hypoxic-ischemia brain damage is one of the major causes of death in the world. There are some plants that have been shown to modify a cerebral ischemia or hypoxia insults. *Pistacia vera* (Anacardiaceae) geographically grows in the Khorassan, Semnan, and Kerman provinces (Iran). Investigations about the other genus of this plant have been shown some pharmacological effects such as reducing blood pressure [1], anti-inflammatory [2, 3] and antimicrobial action [4, 5]. Recently, the antioxidant properties of natural flavonoids and related phenolic compounds extracted from dietary or herb plants have aroused much attention [6]. The plants rich in these compounds are ideal source of natural antioxidants. It has been shown that gallotannin components (pistafolin A), isolated from the leaf extract of *Pistacia weinmannifolia*, have the free radical (FR)

scavenging and protective effects on oxidative neural cell damage. In cerebral granule, cells pretreated with Pistafolin A, peroxynitrite-induced oxidative neural damage and apoptosis were prevented markedly [7].

In recent years, it was shown that the extract of *P. vera* gum has analgesic, anti-inflammatory [8] and hepatoprotective effects against CCl<sub>4</sub>-induced liver toxicity [9]. Recent reports have implied that oxygen FR is important mediators of tissue injuries specially brain in an ischemia-reperfusion sequences. In addition, ischemia causes increased lipid peroxidation reaction and elevates production of FR that contribute to secondary damage to nervous tissue [10, 11]. During ischemia-reperfusion, the cellular membrane severely disrupted and the tissue energy and necessary precursors are failed [12] so that causes disturbance of ionic homeostasis and enzymatic lipolysis [13, 14].

\*Corresponding Author; Fax: (+98-511) 8882 3251, E-mail: hosseinzadehh@yahoo.com

In the light of these bases, we investigated the protective effects of the hydroalcoholic extract of *P. vera* L. gum on lipid peroxidation marker and antioxidant power of hippocampus. Ischemia was induced using four-vessel occlusion model. Lipid peroxidation was assessed by the tissue malondialdehyde (MDA) level (assayed by the thiobarbituric acid [TBA] test) and antioxidant power of the tissue was assessed by ferric reducing-antioxidant power (FRAP) test.

## MATERIALS AND METHODS

**Animals.** Male NMRI rats, weighing 200-250 g, were obtained from a random bred colony maintained with laboratory pellet chow (Khorassan Javane Co., Mashhad, Iran) in Animal House of Mashhad University of Medical Sciences (Iran). Animals were housed in the colony room with 12:12 light/dark cycle at  $21 \pm 2^\circ\text{C}$  and had free access to water and food.

**Plant materials.** *P. vera* L. gum was collected from the south of Khorassan in August 2003. This plant was identified in Herbarium Center of Ferdowsi University (Mashhad, Iran) (012-1622-05). Extraction was conducted by maceration method. Briefly, the powdered *P. vera* gum was extracted with mixture of ethanol and water (3:1) at room temperature for 48 h. The extracts were filtered, the residue was reextracted under the same condition and the combined filtrates were evaporated to dryness at  $40^\circ\text{C}$ . The fraction was dissolved in distilled water just prior to experimentation.

**Preliminary phytochemicals tests.** Phytochemicals screening of the extract was performed using the following reagents and chemicals. Alkaloids with dragendorff's reagent, flavonoids with the use of Mg and HCl; tannins with 1% gelatin and 10% NaCl solutions, and saponins with ability to produce suds [15].

**Surgery.** Ischemia was induced in rat using a four-vessel occlusion model [16]. Briefly, first day, rats were intraperitoneally anesthetized with a mixture of ketamine (60 mg/kg) and xylazine (6 mg/kg) and both vertebral arteries were electrocauterized permanently after exposure to the alar foraminae. The rats were allowed to recover from surgery overnight, and during that time they had

free access to water and food. On the next day, following light anesthesia induced by ketamine/xylazine (30/3 mg/kg, i.p.), a cerebral ischemia was produced by exposure to the carotid arteries and occluding them with microvascular clips for 10 min in all experimental groups [17]. Sham-operated animals underwent both surgical procedures without arterial clamping. Subsequently, both clamps were removed and both arteries inspected for immediate reperfusion. Rats were divided in five groups (six in each group): group A including sham-operated animals and group B served as ischemic control and normal saline (10 ml/kg, i.p.) was injected. The extract of *P. vera* gum (0.1, 0.25, 0.5 g/kg, i.p.) were administered to the remaining three groups. All agents were administered immediately at the onset of reperfusion and every 24 h for 2 consecutive days. After 72 h, the animals were decapitated and their hippocampuses were removed for the biochemical tests.

**MDA assay.** The tissue MDA level was determined based on its reaction with TBA. At the end of the treatment, the brain was removed by fast craniotomy and the hippocampus portion was homogenized with cold 1.5% KCl to make a 10% homogenate. To a 10-ml centrifuge tube, 0.5 ml of 10% homogenate, 3 ml of 1% phosphoric acid and 1 ml of 6% TBA solution were added. The mixture was heated in a boiling water bath for 45 min. After cooling, 4 ml of n-butanol was added and mixed vigorously [18]. After centrifugation, absorption of the supernatant was measured at 532 nm. 1, 1, 3, 3-tetramethoxypropane was used as a standard of MDA. MDA was expressed as nmol per g of the wet tissue.

**Antioxidant power assay.** Antioxidant power of hippocampus was determined by measuring its ability to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  established as the FRAP test [19, 20]. The reagents included 300 mM acetate buffer solution (pH 3.6) with 16 ml acetic acid per 1 liter of buffer solution, 10 ml TPTZ (trypyridyl-s-triazine) in 40 mM HCl and 20 mM  $\text{FeCl}_3$ . Working FRAP reagent was prepared as required by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml  $\text{FeCl}_3$ . Fifty  $\mu\text{l}$  of 10% homogenate was added to 1.5 ml of freshly prepared reagent warmed at  $37^\circ\text{C}$ . After 10 min, the complex between  $\text{Fe}^{2+}$  and TPTZ gives a blue color with absorbance at 593 nm.  $\text{FeSO}_4$  was used as a standard of FRAP assay and data was expressed as  $\mu\text{M}$  per g of wet tissue.

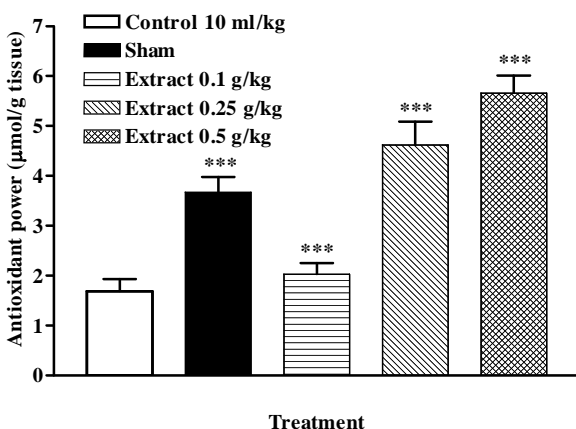
**Statistical analysis.** Data are expressed as mean  $\pm$  S.D. Statistical analysis was performed with one-way ANOVA followed by Tukey-Kramer *post-hoc* test for multiple comparisons. The dose of the extract to produce an effect in 50% of tests (ED<sub>50</sub>) and its associated 95% confidence limits was calculated by Litchfield and Wilcoxon methods (PHARM/PCS Version 4).  $P < 0.05$  was considered statistically significant.

## RESULTS

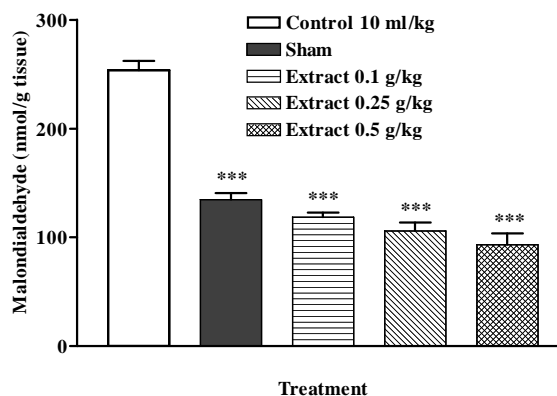
Preliminary phytochemical test indicated that the extract of *P. vera* gum contains flavonoids, tannins, and saponins.

**Effects of the extract on lipid peroxidation.** The MDA level was significantly elevated in brain tissue from subjects in the control group in comparison with those of sham-operated one (47%,  $P < 0.001$ ). The extract of *P. vera* gum at doses of 0.1, 0.25, and 0.5 g/kg decreased brain MDA level by 53% ( $P < 0.001$ ), 58% ( $P < 0.001$ ), and 63% ( $P < 0.001$ ) of control, respectively. Control level of the tissue was  $253.67 \pm 8.4$  nmol/g (Fig. 1). In this test, ED<sub>50</sub> value of the extract was 0.064 g/kg (%95 CL: 0.023, 0.176).

**Effects of the extract on antioxidant power.** Total antioxidant power of hippocampus was significantly decreased from subjects in control group when compared with that of sham-operated animals



**Fig. 1.** Effects of *Pistacia vera* gum extract on brain malondialdehyde (MDA) levels in rats subjected to transient cerebral ischemia. The extract was administered intraperitoneally in different doses of 0.1, 0.25, and 0.5 g/kg. Values are expressed as mean  $\pm$  S.D. of six animals in each group. \*\*\*  $P < 0.001$ . Compared to control, Tukey-Kramer test.



**Fig. 2.** Effects of *Pistacia vera* extract on brain antioxidant power in rats subjected to transient cerebral ischemia. The extract was administered intraperitoneally in different doses of 0.1, 0.25, and 0.5 g/kg. Values are expressed as mean  $\pm$  S.D. of six animals in each group. \*\*\*  $P < 0.001$ . Compared to control, Tukey-Kramer test.

(117%,  $P < 0.001$ ). The extract of *P. vera* gum at doses of 0.25, 0.5 g/kg increased antioxidant power by 173% ( $P < 0.001$ ), 253% ( $P < 0.001$ ) of control, respectively. At dose of 0.1 g/kg, the extract did not significantly modify the antioxidant power of the brain. Control level of the tissue was  $1.69 \pm 0.24$  μmol/g (Fig. 2). The ED<sub>50</sub> was not measurable.

## DISCUSSION

The present data indicate that transient cerebral ischemia induces lipid peroxidation and decrease antioxidant power of hippocampus and are agreed with other studies [23, 24]. Results also indicated that administration of *P. vera* L. gum extract protects hippocampus from ischemia-reperfusion.

Compelling evidence implicates FR as major contributors to ischemic and excitotoxic tissue injury in the CNS. However, the biochemical mechanisms leading to FR production during ischemic brain injury remain partially unclear due to the methodological difficulties in detecting the short-lived FR in the *in vivo* experiments, as well as lack of the neuronal circuitry required for FR generation in the *in vitro* model systems [28]. Organotypic hippocampal slices, which preserve neuronal circuitry and are easily accessible for experimental manipulations, show that FR overproduction in hippocampal pyramidal neurons during ischemia/reoxygenation depends on glutamate receptors activation, and is associated with elevations of intracellular calcium [29]. The mechanisms of calcium-dependent FR generation

caused by ischemia reoxygenation injury might include xanthine/xanthine oxidase reaction, activation of NO, or phospholipids A<sub>2</sub>. Nevertheless, mitochondria are thought to be the principal source of glutamate-mediated, calcium-dependent FR production in cultured cortical neurons [21]. Specifically, n-methyl-d-aspartate receptor-mediated FR generation has been blocked by inhibitors of the mitochondrial electron transport, and mimicked by electron transport uncouplers, suggesting that the glutamate receptor-mediated, (and) calcium-dependent uncoupling of the mitochondrial electron transport could be a major mechanism of FR formation during excitotoxic injury [22].

Results obtained from others species of *Pistacia* indicate that mechanisms including decrease in blood pressure [1], anti-inflammatory [2, 3], antimicrobial action [4, 5], and antioxidant or FR scavenging activity [7] were introduced. In addition, we reported the analgesic, anti-inflammatory [9], and hepatoprotective effect of gum extract of *P. vera* against CCl<sub>4</sub>-induced liver injury [8]. Our phytochemicals studies revealed that the gum extract of *P. vera* have flavonoids, tannins, and saponins. Recently, the antioxidant properties of various flavonoids and related phenolic compounds have been studied extensively, and in addition, antihypoxic and anti-ischemic activities have been reported for some of these components [25-27]. The MDA levels increased significantly following cerebral ischemia-reperfusion injury. The extract reversed the increase of MDA levels to a considerable extent, thereby confirming its antioxidant role in the ischemia. We evaluate the antioxidant or reducing potential of hippocampus homogenate samples following ischemia-reperfusion injury, using FRAP assay. As expected following the reperfusion, the extract increased the antioxidant power of homogenate samples of hippocampus.

In this regard, the results of present study suggested that the neuroprotective effects of the gum extract of *P. vera* may be due to increasing antioxidant defense and suppression FR production in brain.

#### ACKNOWLEDGMENTS

The authors are thankful to the Vice Chancellor of Research, Mashhad University of Medical Sciences (Iran) for financial support.

#### REFERENCES

- Villar, A., Sanz, M.J. and Paya, M. (1987) Hypotensive effects of *Pistacia lentiscus* L. *Int. J. Crud Drug Res.* 25: 1-3.
- Giner, E.M., Manez, S., Recio, C., Giner, R.M. and Prieto, J.M. (2001) Oleanonic acid, a 3-oxotritrepene from *Pistacia* inhibits leukotriene synthesis and has anti-inflammatory activity. *Eur. J. Pharmacol.* 428: 137-143.
- Giner, E.M., Manez, S. and Giner, R.M. (2000) The anti-inflammatory and anti-phospholipase A<sub>2</sub> activity of extract from lanostane-rich species. *J. Ethnopharmacol.* 73: 61-69.
- Ali-Shtayeh, M.S. and Abu Ghdeib, S.I. (1999) Antifungal activity of plant extracts against dermatophytes. *Mycoses* 42: 665-672.
- Magiatis, P., Melliou, E., Shaltsounis, A.L., Chinou, I.B. and Mitaku, S. (1999) Chemical composition and antimicrobial activity of the essential oils of *Pistacia lentiscus* var. Chiao. *Planta Med.* 65: 749-752.
- Hou, A.J., Peng, L.Y., Liu, Y.Z., Lin, Z.W. and Sun, H.D. (2000) Gallotannins and related polyphenols from *Pistacia weinmannifolia*. *Planta Med.* 66: 624-666.
- Weia, T., Sunb, H., Zhaoa, X., Houa, J., Houb, A., Zhaob, Q. and Xina, W. (2002) Scavenging of reactive oxygen species and prevention of oxidative neural cell damage by a novel Gallontamin, Pistafolia A. *Life Sci.* 70: 1889-1899.
- Parvardeh, S., Niapoor, M. and Hosseinzadeh, H. (2002) Hepatoprotective activity of *Pistacia vera* L. gum extract in rats. *Iran. J. Med. Plants.* 1(4): 27-34.
- Parvardeh, S., Niapoor, M., Nassiri Asl, M. and Hosseinzadeh, H. (2002) Antinociceptive, anti-inflammatory and toxicity effects of *Pistacia vera* extract in mice and rat. *Iran. J. Med. Plants.* 1: 59-68.
- Dubinsky, J.M., Kristal, B.S. and Elizondo-Fornier, M. (1995) An obligate role for oxygen in the early stages of glutamate-induced delayed neuronal death. *J. Neurosci.* 15: 7071-7078.
- Patel, M., Day, B.J., Crapo, J.D., Fridovich, I. and McNamara, J.O. (1996) Requirement for superoxide in excitotoxic cell death. *Neuron.* 16: 345-355.
- Weglicki, W.B., Dichens, B.F. and Mak, J.T. (1984) Enhanced lysosomal phospholipid degradation and lysophospholipid production due to free radicals. *Biochem. Biophys. Res. Commun.* 124: 229-235.
- Safar, P. (1988) Resuscitation from clinical death: Pathophysiologic limits and therapeutic potentials. *Crit. Care Med.* 16: 923-941.
- Shapiro, H.M. (1985) Post-cardiac arrest therapy: Calcium entry blockage and brain resuscitation. *Anesthesia.* 62: 384-387.
- Trease, G.E. and Evans, W.C. (1983) Pharmacognosy. Bailliere Tindall Press, London, UK.

16. Pulsinelli, W.A. and Brierley, J.B. (1979) A new model of bilateral hemispheric ischemia in the unanesthetized rat. *Stroke*. 10: 267-272.
17. Suk, K., Kim, S.Y., Leem, K., Kim, Y.O., Park, S.Y., Hur, J., Baek, J., Lee, K.J., Zheng, H.Z. and Kim, H. (2002) Neuroprotection by methanol extract of *Uncaria rhynchophylla* against global cerebral ischemia in rats. *Life Sci*. 70: 2467-2480.
18. Yavuz, O., Turkozkan, N., Bilgihan, A., Dogulu, F. and Aykol, S. (1997) The effect of 2-chloro-adenosine on lipid peroxide level during experimental cerebral ischemia-reperfusion in gerbils. *Free Rad Biol. Med*. 22: 337-341.
19. Benzie, I.F. and Strain, J.J. (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem*. 239:70-76.
20. Abdollahi, M., Mostafalou, S., Pourmohammadi, S. and Shadnia, S., (2004) Oxidative stress and cholinesterase inhibition in saliva and plasma of rats following subchronic exposure to malathion. *Compar. Biochem. Physiol*. 137: 29-34.
21. Fisher, M. (2001) Stroke therapy. In: *Pathophysiology of Ischemic Injury*. (Ahmed, S.H., Hu, C.J., Paczynski, R. and Hsu, C.Y. eds.), Buffer Worth-Heinemann, USA. pp. 25-57.
22. Dugan, L.L. and Choi, D.W. (1999) Hypoxic-ischemic brain injury and oxidative stress. In: *Basic Neurochemistry: Molecular, Cellular and Medical Aspects*. (Siegel, G.J., Agranoff, B.W., Albers, R.W., Fischer, S.K. and Uhler, M.D. eds.), Lippincott-Raven Publishers, Philadelphia, pp.711-729.
23. Hall, E.D. (1997) Brain attack. acute therapeutic interventions. Free radical scavengers and antioxidants. *Neurosurg. Clin. N. Am*. 8: 192-206.
24. Calapai, G., Squadrito, F., Rizzo, A., Crisafulli, C., Campo, G.M., Marciano, M.C., Mazzaglia, G. and Caputi, A.P. (1993) A new antioxidant drug limits brain damage induced by transient cerebral ischemia. *Drugs Exp. Clin. Res*. 19: 159-164.
25. Calapai, G., Crupi, A., Firenzuoli, F., Marciano, M.C., Squadrito, F., Infereera, G., Parisi, A., Rizzo, A., Crisafulli, C., Fiore, A. and Caputi, A.P. (2000) Neuroprotective effects of *Ginkgo biloba* extract in brain ischemia are mediated by inhibition of nitric oxide synthesis. *Life Sci*. 67: 2673-2683.
26. Dou, D.Q., Zhang, Y.W., Zhang, L., Chen, Y.J. and Yao, X.S. (2001) The inhibitory effects of ginsenosides on protein tyrosine kinase activated by hypoxia/reoxygenation in cultured human umbilical vein endothelial cells. *Planta Med*. 67: 19-23.
27. Nishimura, S., Taki, M., Takaishi, S., Iijima, Y. and Akiyama, T. (2000) Structures of 4-aryl-coumarin (neoflavone) dimers isolated from *Pistacia chinensis* BUNGE and their estrogen-like activity. *Chem. Pharm. Bull*. 48: 505-508.
28. Frantseva, M.W., Carlen, P.L. and Velazquez, J.A. (2001) Dynamics of intracellular calcium and free radical production during ischemia in pyramidal neurons. *Free Radical Biol. Med*. 31: 1216-1227.
29. Velazquez, J.L., Frantseva, M.V. and Carlen, P.L. (1997) *In vitro* ischemia promotes glutamate-mediated free radical generation and intracellular calcium accumulation in pyramidal neurons of cultured hippocampal slices. *J. Neurosci*. 17: 9085-9094.