Beneficial Effects of Hippophae rhamnoides L. on Nicotine Induced Oxidative Stress in Rat Blood Compared with Vitamin E

Halis Suleyman, a Kenan Gumustekin, b Seyithan Taysi, c Sait Keles, b Nuray Oztasan, b Omer Aktas, b Konca Altinkaynak, c Handan Timur, c Fatih Akcay, c Sedat Akar, b Senol Dane, b and Mustafa Gul a,b

a Department of Pharmacology, Faculty of Medicine, Ataturk University; b Department of Physiology, Faculty of Medicine, Ataturk University; and c Department of Biochemistry, Faculty of Medicine, Ataturk University; 25240, Erzurum, Turkey. Received April 1, 2002; accepted May 13, 2002

The aim of this study was to determine the effects of Hippophae rhamnoides L. extract (HRE-1) and also vitamin E as a positive control on nicotine-induced oxidative stress in rat blood, specifically alterations in erythrocyte malondialdehyde (MDA) level, activities of some erythrocyte antioxidant enzymes, and plasma vitamin E and A levels. The groups were: nicotine (0.5 mg/kg/d, intraperitoneal, i.p.); nicotine+vitamin E (75 mg/kg/d, intragastric, i.g.); nicotine+HRE-1 (1 ml/kg/d, i.g.); and control group (receiving only vehicles). There were 8 rats per group and the supplementation period was 3 weeks. Nicotine-induced increase in erythrocyte MDA level was prevented by both HRE-1 and vitamin E. Nicotine-induced decrease in erythrocyte superoxide dismutase (SOD) activity was prevented by HRE-1, but not vitamin E. HRE-1 increased the erythrocyte glutathione peroxidase (GSH-Px) activity compared with nicotine and the vitamin E groups. Catalase activity was not affected. Vitamin E supplementation increased plasma vitamin E level. Plasma vitamin A level was higher in both vitamin E and HRE-1 supplemented groups compared with nicotine and control groups. The results suggest that HRE-1 extract can be used as a dietary supplement, especially by people who smoke, in order to prevent nicotine-induced oxidative stress.

Key words Hippophae rhamnoides L.; nicotine; oxidative stress; vitamin E; antioxidant enzyme; malondialdehyde

It is known that nicotine, a major toxic component of cigarette smoke, induces oxidative stress both in vitro and in vivo. Increased free radical production and cooperative lipid peroxidation levels in pancreatic tissue of rats incubated with nicotine, and increased lipid peroxidation in Chinese hamster ovary cells incubated with nicotine and smokeless tobacco extract have been reported. In addition, elevated oxidative DNA damage in various tissues of mice exposed to side-stream cigarette smoke, and increased lipid peroxidation levels in tissues of intraperitoneal nicotine administered rats have been found. Furthermore, increased lipid peroxidation in blood of smokers has also been reported. It seems that people who smoke and also who are exposed to cigarette smoke indirectly by breathing the air in the same environment are exposed to nicotine-induced oxidative stress.

On the other hand, Basier et al. have reported that smokers consume fewer green vegetables and fruits, which are rich in antioxidants, than non-smokers of both sexes. Differences in dietary habits may also exacerbate the nicotine-induced oxidative stress in smokers.

Oxidative stress is considered to take part in the pathogenesis of various diseases, including cancer, diabetes, and cardiovascular diseases. Thus, nicotine-induced oxidative stress may play an important role in the development of cardiovascular disease and lung cancer in smokers.

Hippophae rhamnoides L., a member of the Elaeagnaceae family, is a perennial plant native to Europe and Asia and also widely distributed in the fields of north and east Anatolia. Its fruits are orange colored, sour to the taste, single-seeded and 3—7 mm in diameter and contain carotenes (α, β, δ), vitamins C, E, riboflavin, folic acid, tannins, sugar, glycerides of palmitic, stearic and oleic acids, polyphenols and some essential amino acids. Fruits of Hippophae rhamnoides L. have been used extensively in traditional medicine in Turkey as well as China and former Soviet Republics to treat constipation, gastric ulcer, skin wounds and influenza infections. Beneficial affects of HRe-1 have been shown in experimental gastric ulcer models. Antioxidant activity of Hippophae rhamnoides L. has been shown in vitro, cell culture and animal studies. Different fractions of sea buckthorn (Hippophae rhamnoides L.) fruits inhibit 2,2-azobis (2,4-dimethylvaleronitrile) and ascorbate-iron induced lipid peroxidations in vitro. Hippophae rhamnoides, as well as vitamin E, decreases the malondialdehyde (MDA) content in hyperlipidemic rabbit serum cultured smooth muscle cells. Seed oil of Hippophae rhamnoides L. inhibits MDA formation of liver induced by CCl4, acetaminophen and ethyl alcohol and also prevents the acetaminophen-induced glutathione depletion in liver. The prevention of glutathione depletion by HRE-1 is also reported in gastric tissue in ethanol administered rats. However, no study has reported the effects of HRE-1 on nicotine-induced lipid peroxidation.

Vitamin E, the term for a group of tocopherols and tocotrienols, is well accepted as nature’s most effective lipid-soluble, chain-breaking antioxidant, protecting cell membranes from peroxidative damage. Studies show that vitamin E also counteracts against nicotine-induced lipid peroxidation in animals and also humans.

Hence, the aim of this study was to determine the effects of HRE-1 and vitamin E supplementation as a positive control on nicotine-induced oxidative stress in rat blood, specifically alterations in erythrocyte MDA level, activities of some erythrocyte antioxidant enzymes, and plasma vitamin E and A levels. The results of the study may have clinical implications since HRE-1 is a non-toxic plant extract that can easily...
be used as a dietary supplement.

MATERIALS AND METHODS

Animals  Thirty-two rats (Sprague-Dawley strain with a body weight of 225±28 g), fed with standard laboratory chow and water, were used in the study. They were randomly divided into 4 groups (8 rats per group) and placed in separate cages during the study. The groups were as follows:

- Group I: nicotine (0.5 mg/kg/day, i.p.)
- Group II: nicotine (0.5 mg/kg/day, i.p.) + vitamin E (75 mg/kg/day, i.g.)
- Group III: nicotine (0.5 mg/kg/day, i.p.) + HRe-1 (1 ml/kg/day, i.g.)
- Group IV: control group (received only the same amounts of vehicles, 0.9% NaCl solution, i.p., and corn oil, i.g.).

Supplementation period was 3 weeks. Animal experiments were carried out in an ethically proper way by following guidelines as set by the Ethical Committee of the Ataturk University.

Preparation and Administration of Hippophae rhamnoides L. Extract  The ripe fresh fruit of Hippophae rhamnoides L. were collected from the Tortum area (altitude of 1600 m), a town in Erzurum, Turkey. The plant was identified by Dr. Ali Aslan from the Department of Pharmaceutical Botany, Faculty of Pharmacy, Ataturk University, Turkey. Fruits of Hippophae rhamnoides L. were removed from the branches, washed with tap water and dried, then crushed in a mortar and mixed. Fruit mash was placed in a glass jar and hexane was added in equal volume. Forty-eight hours later, juice was obtained from the mixture by squeezing and centrifuging at 1000×g for 15 min; clear supernatant was removed by a drip. Hexane was evaporated from the liquid by an evaporator (Büchi, Rotavapor, R110, Switzerland). Hippophae rhamnoides L. extract (HRe-1) was also mixed with corn oil (1/1, v/v), and administered orally by a stomach tube to group 3 for 3 weeks at 1 ml/kg/d.

Preparation and Administration of Nicotine  Hydrogen tartrate salt of nicotine (Sigma N-5260) was dissolved in 0.9% NaCl solution to get a 0.15 mg/ml concentration of nicotine. Then, pH of the nicotine solution was adjusted to 7.4 by 0.1 N NaOH. Nicotine (0.5 mg/kg/d) was administered by intraperitoneal injection to groups 1, 2 and 3 for 3 weeks.

Preparation and Administration of Vitamin E  Vitamin E (Ephynal 300 capsule, Roche, France) was dissolved in corn oil (30 mg/ml) and administered orally by a stomach tube (approximately 75 mg/kg/d) to group 2 for 3 weeks.

Determination of Erythrocyte MDA Level, and the Activities of SOD, Catalase and Glutathione Peroxidase (GSH-Px)  At the end of the experiment, the animals were anesthetized with ketamine–HCl (Ketalar, 20 mg/kg, i.p.), and the blood was collected by cardiac puncture after thoracotomy. Blood samples were collected in vacutainer tubes and the blood was collected by cardiac puncture after thoracotomy. Blood samples were collected in vacutainer tubes and the blood was collected by cardiac puncture after thoracotomy. Blood samples were collected in vacutainer tubes and the blood was collected by cardiac puncture after thoracotomy. Blood samples were collected in vacutainer tubes and the blood was collected by cardiac puncture after thoracotomy.

RESULTS

Nicotine increased the erythrocyte MDA level compared with the control group. This nicotine-induced increase was prevented in both vitamin E and HRe-1 supplemented nicotine-administered groups (Fig. 1).

Nicotine decreased the activity of erythrocyte SOD compared with the control group (Fig. 2A). This nicotine-induced decrease in activity was prevented by HRe-1, but not vitamin E (Fig. 2A). Nicotine plus HRe-1 increased the erythrocyte GSH-Px activity compared with the nicotine and the nicotine plus vitamin E groups, however, neither nicotine
alone nor nicotine plus vitamin E affected the erythrocyte GSH-Px activity (Fig. 2B). Of the erythrocyte antioxidant enzymes studied, catalase activity was not affected by any of the treatments (data not shown).

Vitamin E supplementation increased plasma vitamin E level compared with control and nicotine-administered groups (Fig. 3A). Plasma vitamin A levels in both vitamin E and HRe-1 supplemented groups were higher than control and nicotine administered groups (Fig. 3B).

DISCUSSION

To our knowledge, prevention of nicotine-induced oxidative stress by HRe-1 in erythrocytes of nicotine-administered rats is reported here for the first time.

Our result, increased oxidative stress determined as a higher erythrocyte MDA level in nicotine-administered rats, agrees with studies showing increased MDA levels in blood and serum of smokers, elevated 8-hydroxy-2'-deoxyguanosine levels showing oxidative DNA damage in heart, lung, and liver tissues of mice exposed to side-stream cigarette smoke, increased TBARS, conjugated diene and hydroperoxide levels in heart, liver and lung tissues of intraperitoneally nicotine-administered rats. It is also shown that addition of free radical scavenging enzymes, SOD and catalase, prevent nicotine-induced increase in lipid peroxidation in pancreatic tissue of rat and decrease in cellular glutathione level in Chinese hamster ovary cells. The results of these studies suggest that superoxide anions and hydrogen peroxide are the main source of nicotine-induced free radical production depleting the cellular glutathione level. In agreement with the decreased SOD activity found in various rat tissues, decreased erythrocyte SOD activity would have taken part in the nicotine-induced oxidative stress in the present study.

In contrast to the alterations in activity levels of antioxidant enzymes due to nicotine and vitamin E supplementation in our study, increased erythrocyte cytosolic antioxidant enzyme activities in smokers compared with non-smokers, and
further increase in erythrocyte catalase activity but a decrease in erythrocyte SOD activity due to a 10 week vitamin E supplementation in smokers have also been reported\(^{22}\).

HRe-1 supplementation prevented the nicotine-induced decrease in erythrocyte SOD activity and also remarkably increased erythrocyte GSH-Px activity in nicotine-treated rats, which would have contributed to the prevention of nicotine-induced increase in erythrocyte MDA level. It seems that alterations of antioxidant enzyme activities in response to nicotine-induced lipid peroxidation and also antioxidant supplements such as vitamin E and *Hippophae rhamnoides* L. are complicated.

In our study, nicotine-induced increase in erythrocyte MDA level was prevented by vitamin E as well as HRe-1 supplementation. Erythrocyte GSH-Px activity was remarkably increased by HRe-1 compared to the nicotine and the nicotine plus vitamin E groups. Nicotine-induced decrease in erythrocyte SOD activity was prevented by HRe-1, but not by vitamin E. Remarkable increase in erythrocyte GSH-Px activity, and the increase in plasma vitamin A level in HRe-1-supplemented group may have played a role in the prevention of nicotine-induced oxidative stress in our study. GSH-Px takes part in detoxification of hydrogen peroxide by converting it to water and molecular oxygen using glutathione as substrate.\(^{19}\)

Increased plasma vitamin E and A levels may be responsible for the prevention of nicotine-induced lipid peroxidation in vitamin E-supplemented group. Vitamin E supplementation can explain the increase in plasma vitamin E level. Parallel to our finding, a higher level of vitamin A in liver tissue of vitamin E-supplemented rats has also been reported by Helen *et al.*\(^{19}\) It is possible that vitamin E supplementation might have increased the absorption of vitamin A from the intestine. The increase in plasma vitamin A level in the HRe-1-supplemented group may be explained by carotenes present in the HRe-1 extract.\(^{1136}\)

In conclusion, HRe-1, as well as vitamin E, prevented the increase in oxidative stress in erythrocytes of nicotine-administered rats. Remarkable increase in erythrocyte GSH-Px activity, and the increase in plasma vitamin A level in HRe-1 supplemented group might have contributed to the prevention of nicotine-induced oxidative stress. The results of this study suggest that HRe-1 extract can be used as a dietary supplement, especially by people who smoke, in order to prevent nicotine-induced oxidative stress.

**Acknowledgements** This study was partly supported by The Research Foundation of Ataturk University, Erzurum, Turkey (project no: 2001/53). The authors thank Delali Camgöz, who has taken care of the animals.

**REFERENCES**


15) Turova A. D., Sapojnikova E. N., “Herbal Medicines and Their Usage in Russia,” (Lekarstvennie rasteniye SSSR i ih primeneniye) Moscow. 1982. (in Russian)


