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Protective effects of rutin and chlorogenic acid against antihypoxic conditions in mice

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Abstract: Almost all plants contain polyphenols. Literature shows that polyphenols exhibit many biological activities. Little has known about their protective effects against hypoxia-induced lethality. The protective effects of rutin (1) and chlorogenic acid (2) against hypoxia conditions in mice were determined by three different experimental models. Antihypoxic activity was especially pronounced in asphytic hypoxia. Both compounds (1&2) showed statistically significant ($p>0.05$) activities respect to the control. Compound (1) significantly prolonged the latency for death with respect to control (39.20±8.70 vs. 13.20±2.58min, $p<0.001$). Compound (1) was the most effective compound in circulatory hypoxia. It significantly prolonged the latency for death with respect to control (14.44±2.82 vs. 9.82±0.79 min, $p<0.01$). On the other hand, Chlorogenic acid (2) at a dose of 100 mg kg^{-1} kept mice alive for 12.76±1.30min ($p>0.05$). None of two phenolic acids had any activity in haemic hypoxia when compared to control.

Keywords: Antihypoxia, asphyctic hypoxia, rutin.

INTRODUCTION

The imbalance between low oxygen supply and oxygen demands determines formation of hypoxia in different organs. It occurs especially in heart attack, heart diseases, ischemia and causing many deleterious effects and finally death (Kiang and Tsen, 2006). Hypoxia causes oxidative stress involving production of reactive oxygen species (ROS) (Maiti *et al.,* 2006). It has proven that the compounds with antioxidant activity can scavenge ROS and able to exhibit antihypoxic property. There are increasing interests in using natural antioxidants instead of the chemical compounds. Polyphenolic compounds are receiving increased attention as epidemiological studies. Rutin (fig. 1, compound 1) is a natural flavonoid and abundantly present in vegetables, tea and red wine (Nabavi *et al.,*2012). Compound (1) has a wide range of biological activities such as anti-inflammatory, anti-ulcer, immunomodulatory, vasodilator, hepatoprotective, anti-hypertensive, cardioprotective and neuroprotective properties (La Casa *et al.,* 2000; Janbaz *et al.,* 2002). These beneficial effects of rutin are due to its high radical scavenging activity and antioxidative capacity (Korkmaz and Kolankaya, 2010). Chlorogenic acid (fig. 1, compound 2) is a phenolic product naturally isolated from the leaves and fruits of plants, including the coffee bean and green tea leaves, peach, apples, pears and berries. It is one of the most plentiful dietary polyphenols. It has various biological properties such as antioxidant, anticarcinogenic, antidiabetic, anti-hypertensive, antinociceptive and inflammatory activities (Santos *et al.,* 2006; Zhao *et al.,* 2012). Compound (2) has shown a significant improvement in oxidative stress in rats (Koriem and Soliman, 2014). We have recently reported antihypoxic activities of *Cantharellus cibarius* (Khalili *et al.,* 2014), *Eryngium caucasicum* and *Urtica dioica* (Khalili *et al.,* 2015). In spite of many published papers, nothing is known about protective effects of rutin (1) and chlorogenic acid (2) against hypoxia-induced lethality. These two compounds are reported as constituents of these plants. The aim of the present work was to screen the anti-hypoxic activities of two known phenolic compounds in order to understand a possible mechanism of their action in cardiovascular diseases.

MATERIALS AND METHODS

Chlorogenic acid and rutin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Male Swiss albino mice (25±2g) were randomly housed in groups of 10 in polypropylene cages at an ambient temperature, 25±1°C and 45-55% relative humidity, with a 12h dark: 12h light cycle (lights on at 8 a.m.). The animals had free access to standard food and water and *libitum*. Experiments were conducted between 9:00 and 14:00h. All the experimental procedures were conducted in accordance with the NIH guidelines of the Laboratory Animal Care and Use. The Institutional Animal Ethical Committee of Mazandaran University of Medical Sciences also approved the experimental protocol.

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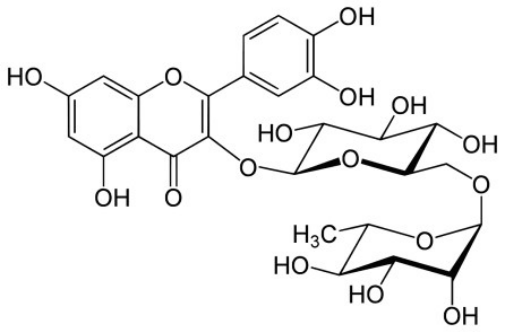
Asphyctic hypoxia

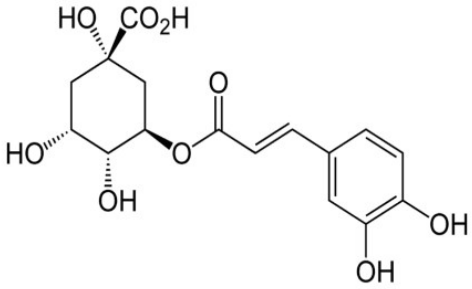
In this method, the animals were putting individually in a firmly closed 300 ml glass container which was placed under water in an aquarium of 25ºC. The animals had convulsions and died from hypoxia. The latencies for death were recorded. The animals died approximately 2min following convulsions. Mice received single i.p. injections of 10, 20 and 100mg kg^{-1} doses of rutinor chlorogenic acidor phenytoin (50mg kg^{-1}) as 30min before they were subjected to hypoxia. Another control group was treated with normal saline (Eslami *et al.,* 2011).

Table 1: Antihypoxic activities of rutin and chlorogenic acid in haemic hypoxia in mice.

Groups	Dose (mg/kg)	Haemic hypoxia activity (min)
Control	--	7.40 ± 1.025
Rutin	20	7.01 ± 0.35 ^{ns}
	100	7.50 ± 1.11^{ns}
Chlorogenic acid	20	7.20 ± 0.27 ^{ns}
	100	7.56 ± 0.63 ^{ns}



Rutin (1)



Chlorogenic acid (2)

Fig. 1. Chemical structures of rutin (left) and chlorogenic acid (right).

Haemic hypoxia

Fortymice were divided into five groups each containing eight mice. Normal saline was injected to control group. Thirty minutes after *i.p.* administration of 20 and 100mg kg^{-1} doses of rutinor chlorogenic acid,NaNO (360mg kg^{-1}) was injected *i.p.* to mice and antihypoxic activity was estimated as the latent time of evidence of hypoxia in minutes (Ebrahimzadeh *et al.,* 2010).

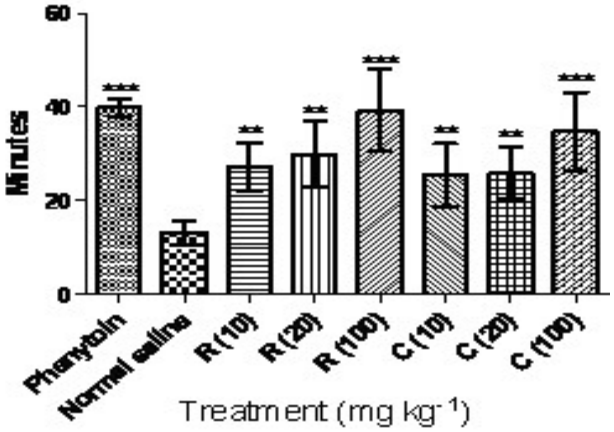


Fig. 2: Antihypoxic activities of rutin (1) and chlorogenic acid (2) in asphyctic hypoxia in mice. Data are expressed as mean ± *SD* (*n* = 8), (**P<0.01, ***P<0.001, compared to control).

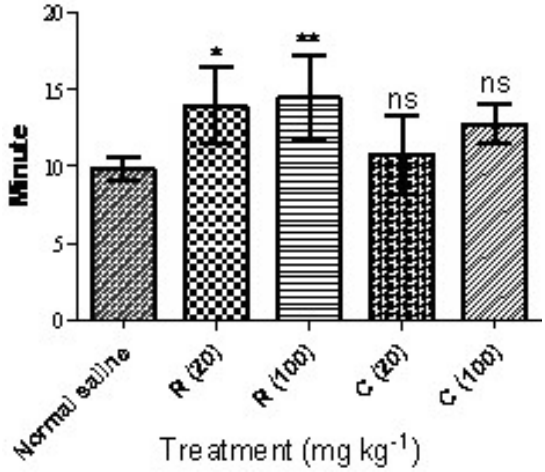


Fig. 3: Antihypoxic activities of rutin (1) and chlorogenic acid (2) in circulatory hypoxia in mice. Data are expressed as mean ± *SD* (*n*=8), (ns, not significant; *P<0.05, **P<0.01, compared to control).

Circulatory hypoxia

Forty mice were divided into five groups each containing eight mice. The groups were treated with normal saline. Thirty minutes after *i.p.* administration of 20 and 100mg kg^{-1} doses of rutinor chlorogenic acid, NaF (150mg kg^{-1}) was injected *i.p.* to mice and antihypoxic activity was measured in minutes as the latent time of evidence of hypoxia (Ebrahimzadeh *et al.,* 2010).

STATISTICAL ANALYSIS

Data were presented as mean ± SD. One way analysis of variance (ANOVA) was performed. Duncan's new multiple-range test was used to determine the differences in means. All *p* values less than 5% were considered as significant.

RESULTS

Statistically significant antihypoxic activities were recognized in some doses of rutin and chlorogenic acid in some experimental model of asphytic, circulatory and haemichypoxia in mice. The results of asphytic hypoxia have been shown in fig. 2. The effects of extracts were dose-dependent. Both compounds, at all tested doses, showed statistically significant activity respect to the control. Rutin (1) 100mgkg^{-1} was the most effective compounds. It significantly prolonged the latency for death with respect to control group (39.20±8.70 vs. 13.20±2.58min for control groups, $p<0.001$). At 10 and 20mgkg^{-1}, it also prolonged survival time (27.10±5.10 and 29.80±6.97min, $p<0.01$ respect to control). Chlorogenic acid (2) at 100mgkg^{-1} also kept mice alive for 34.74±8.44min. This effect was statistically significant from the control ($p<0.001$), too. At 20mgkg^{-1}, it prolonged survival time (25.72±5.54 min, $p<0.01$). Phenytoin prolonged survival time to 39.80±1.92min ($p<0.001$). Extracts showed no activity in haemic model (table 1). Mice in control group died of hypoxia in 7.40±1.025min. None of two phenolic acids had any activity in haemic hypoxia when compared to control. Rutin and Chlorogenic at 100mgkg^{-1} prolonged latency for death to 7.50±1.11 and 7.56±0.63min, but this activity was not statistically significant from control group ($p>0.05$). The results of circulatory hypoxia are shown in fig. 3. Rutin at 100mgkg^{-1} was the most effective compound. It significantly prolonged the latency for death with respect to control group (14.44±2.82 vs. 9.82±0.79min for control groups, $p<0.01$). This effect was dose-dependent. At 20mgkg^{-1}, it prolonged survival time (13.92±2.49min, $p<0.05$). Chlorogenic acid at 100mgkg^{-1} also kept mice alive for 12.76±1.30min. But this effect was not statistically significant from the control group ($p>0.05$).

DISCUSSION

Hypoxia produces a strong physiologic stress and induces a wide range of deleterious effects at the cellular level. The brain, which consumes a large quantity of oxygen, is very vulnerable to low levels of oxygen (Warner *et al.,* 2004). Free radicals act as signaling species in a variety of normal physiological processes. Excessive production of these radicals causes damage to biological material and is an essential event in the pathogenesis of different diseases (Bakony and Radak, 2004).The increased level of ROS in hypoxia is the result of the accumulation of reduction equivalents in the mitochondrial electron transport system due to the lower level of oxygen. The accumulated electrons form super oxide radical anion, which leads to H_2O_2 and hydroxyl radicals (Bakony and Radak, 2004; Halli well, 2006). The effects of ROS can be mainly evident in certain tissues such as brain. Because the brain consumes about 1/5 of the basal oxygen, more than other tissues also undergoing mitochondrial respiration, the potential for ROS exposure is increased. The brain has high content of polyunsaturated fatty acids, which easily undergo oxidation. In addition, the presence of redox-active metals (Cu, Fe) increases the ROS level (Halliwell, 2006). Many efforts have been undertaken to develop therapies to reduce the effects of oxidative stress. Considerable evidence shows that antioxidants can exert protecting action on a variety of illnesses such as cardiovascular and neurodegenerative diseases. There are many investigations, which suggest that polyphenolic compounds are powerful antioxidants and metal chelators. They have a broad spectrum of pharmacological and therapeutic effects (Spencer, 2010).

Statistically significant antihypoxic activities were recognized in some doses of rutin and chlorogenic acid in some experimental model of hypoxia in mice. Both compounds, at all tested doses, showed statistically significant activity respect to the control. Rutinat 100mgkg^{-1} was the most effective compounds. It significantly prolonged the latency for death with respect to control group ($p<0.001$). At lower doses, it also prolonged survival time ($p<0.01$). Chlorogenic acid at 100mgkg^{-1} also kept mice alive for 34.74±8.44min ($p<0.001$), too. At 20mgkg^{-1}, it prolonged survival time (25.72± 5.54min, $p<0.01$) (fig. 2).

Compounds showed no activity in haemic model (table 1). Mice in control group died of hypoxia in 7.40±1.025min. None of two phenolic acids had any activity in haemic hypoxia when compared to control. Rutin and chlorogenic acid at 100mgkg^{-1} prolonged latency for death but this activity was not statistically significant from control group ($p>0.05$). There are some reports about cell protective effects of rutin after ischemic injury of organs, including the brain, heart and kidney (Khan *et al.,* 2009; Korkmazand Kolankaya, 2010). In particular, administration of rutin before transient cerebral ischemia or at the onset of reperfusion has shown a reduction of ischemic neural apoptosis by increasing endogenous antioxidant enzymatic activities in experimental animals (Khan *et al.,* 2009). Rutin might be useful in clinical trials aimed to improve the outcome of patients suffering from acute ischemic stroke (Jang *et al.,* 2014). Animal studies show that dietary rutin supplementation has beneficial effects against liver injury in ischemia/reperfusion (Acquaviva *et al.,* 2009). The results of circulatory hypoxia are shown in fig. 3. Rutin at 100mgkg^{-1} was the most effective compound. It significantly prolonged the latency for death with respect to control group ($p<0.01$). This effect was dose-dependent. At 20mgkg^{-1}, it prolonged survival time (13.92±2.49min, $p<0.05$). Chlorogenic acid at 100mgkg^{-1} also kept mice alive but this effect was not statistically significant from the control ($p>0.05$). There are some published papers that showed administration of sodium

fluoride, substance that induces circulatory hypoxia, will increase the blood histamine content and decrease the capacity of oxygen transport.

An established relationship between oxidative metabolism and cholinergic function has been demonstrated during the study of sodium nitrite ($NaNO_2$) on brain metabolism (Gibson *et al.,* 1978). Chemical hypoxia is induced by the injection of sodium nitrite ($NaNO_2$: 360mgkg^{-1}i.p.), which reduces the oxygen carrying capacity of the blood cells by converting hemoglobin to methemoglobin. This lethal dose (100% of controls) is injected 30min after treatment of extract. Immediately after the $NaNO_2$ injection, the animals are placed in small cages and the time between injection of $NaNO_2$ and cessation of respiration is recorded. The results of haemic hypoxia are shown in table 1. Extracts showed no activity in haemic model (table 1). None of two phenolic acids had any activity in haemic hypoxia when compared to control. Rutin and Chlorogenic at 100mgkg^{-1} prolonged latency for death but this activity was not statistically significant from control group ($p>0.05$). Because there is no standard drug for haemic and circulatory hypoxic models, results of this study were compared to those of control groups. A significant protective effect on other forms of hypoxia such as hypobaric hypoxia has been reported by Ginkgo biloba that contains flavonoids (Ma *et al.,* 2008). Our results may be supported by other published data that show flavonoids increase cerebral blood flow and haveantihypoxic activity (Hertog *et al.,* 1993; He *et al.,* 2010). The mechanism of this protective action may be due in part to the antioxidant activity of flavonoids.

CONCLUSION

Polyphenolic compounds are valuable sources of antihypoxic activity. Rutin and chlorogenic acid showed a reasonable protective effect against the hypoxia in some model. Specifically, they produced significant and dose-dependent effect on the model of asphytic hypoxia. The presence of these polyphenols in some medicinal plants may be a proposal mechanism for reported antihypoxic activities of these plants. The bioactive phytochemicals like rutin and chlorogenic acid are of enormous interest and can be utilized sustainably for discovery of novel therapeutic agents against hypoxic conditions.

REFERENCES

Acquaviva R, Lanteri R, Destri GL, Caltabiano R, Vanella L, Lanzafame S, Cataldo AD, Volti GL and Giacomo CD (2009). Beneficial effects of rutin and Larginine coadministration in a rat model of liver ischemia–reperfusion injury. *Am. J. Physiol. Gastrointest. Liver Physiol.,* **296**(1): G664-G670.

Bakony T and Radak Z (2004). High altitude and free radicals. *J. Sports Sci. Med.,* **3**(2): 64-69.

Dos Santos MD, Almeida MC, Lopes NP and de Souza GEP (2006). Evaluation of the antiinflammatory analgesic and antipyretic activities of the natural polyphenol chlorogenic acid. *Biol. Pharm. Bull.,* **29**(11): 2236-2240.

Ebrahimzadeh MA, Nabavi SF, Nabavi SM and Eslami B (2010). Antihypoxic and antioxidant activity of *Hibiscus esculentus* seeds. *Grasas Aceites,* **61(4)**: 30-36.

Eslami B, Nabavi SF, Nabavi SM, Ebrahimzadeh MA and Mahmoudi M (2011). Pharmacological activities of *Hypericum scabrum* L. *Eur. Rev. Med. Pharmacol. Sci.,* **15**(1): 532-537.

Gibson GE, Shimada M and Blass JP (1978). Alterations in acetylcholine synthesis and cyclic nucleotides in mild cerebral hypoxia. *J. Neurochem.,* **31**(2): 757-760.

Janbaz KH, Saeed SA and Gilani AH (2002). Protective effect of rutin on paracetamol and CCl_4-induced hepatotoxicity in rodents. *Fitoterapia,* **73**(3): 557-563.

Jang J, Lee J, Hur H, Kim T, Joo S and Piao M (2014). Rutin improves functional outcome via reducing the elevated matrix metalloproteinase-9 level in a photothrombotic focal ischemic model of rats. *J. Neurol. Sci.,* **339**(2): 75-80.

Halliwell B (2006). Oxidative stress and neuro-degeneration: Where are we now? *J. Neurochem.,* **97**: 1634-1658.

He C, Ji X, Pan Y, Wang H, Wang K, Liang M and Yang L (2010). Antioxidant activity of alcoholic extract of *Agrimonia pilosa* Ledeb. *Med. Chem. Res.,* **19**(3): 448-461.

Hertog MG, Feskens EJ, Hollman PC, Katan MB and Kromhout D (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study. *Lancet,* **342**(3): 1007-1011.

Kiang JG and Tsen K (2006). Biology of hypoxia. *Chinese J. Physiol.,* **49**(2): 223.

Koriem Kh and Soliman RE (2014). Chlorogenic and caftaric acids in liver toxicity and oxidative stress induced by methamphetamine. *J. Toxicol.,* 2014: Article ID 583494.

Khalili M, Ebrahimzadeh MA, Omrani F and Karami M (2014). Antihypoxic activities of the Golden Chanterelle Mushroom, *Cantharellus cibarius* (Higher Basidiomycetes). *Int. J. Med. Mushrooms,* **16**(4): 339-344.

Khalili M, Dehdar T, Hamedi F, Ebrahimzadeh MA and Karami M (2015). Antihypoxic activities of *Eryngium caucasicum* and *Urtica dioica. Eur. Rev. Med. Pharmacol. Sci.,* **19**(1): 3282-3285.

Khan MM, Ahmad A, Ishrat T, Khuwaja G, Srivastawa P, Khan MB, Raza SS, Javed H, Vaibhav K, Khan A and Islam F (2009). Rutin protects the neural damage induced by transient focal ischemia in rats. *Brain Res.,* **129**(2): 123-135.

Korkmaz A and Kolankaya D (2010). Protective effect of rutin on the ischemia / reperfusion induced damage in

rat kidney. *J. Surg. Res.,* **16**(4): 309-315.

La Casa C, Villegas I, de la Lastra CA, Motilva V and Calero MJ (2000). Evidence for protective and antioxidant properties of rutin a natural flavone against ethanol induced gastric lesions. *J. Ethnopharmacol.,* **71**(4): 45-53.

Ma YQ, Ye XQ, Hao YB, Xu GN, Xu GH and Liu DH (2008). Ultrasound-assisted extraction of hesperidin from Penggan (*Citrus reticulata*) peel. Ultrason. *Sonochem.,* **15**(3): 227-232.

Maiti P, Singh SB, Sharma AK, Muthuraju S, Banerjee PK and Ilavazhagan G (2006). Hypobaric hypoxia induces oxidative stress in rat brain. *Neurochem. Int.,* **49**(2): 709-716.

Nabavi SM, Nabavi SF, Eslami Sh and Hajizadeh Moghaddam A (2012). *In vivo* protective effects of quercetin against sodium fluoride-induced oxidative stress in the hepatic tissue. *Food Chem.,* **13**(2): 931-935.

Spencer JPE (2010). Beyond antioxidants: The cellular and molecular interactions of flavonoids and how these underpin their actions on the brain. *Proc. Nutr. Soc.,* **69**(6): 244-260.

Warner DS, Sheng H and Batinic-Haberle I (2004). Oxidants antioxidants and the ischemic brain. *J. Exp. Biol.,* **20**(7): 3221-3231.

Zhao Y, Wang J, Ballevre O, Luo H and Zhang W (2012). Antihypertensive effects and mechanisms of chlorogenic acids. *Hypertens. Res.,* **35**(4): 370-37.