



## PROTECTIVE EFFECTS OF *VICIA HIRSUTA* AGAINST HYPOXIA-INDUCED LETHALITY IN MICE

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### ABSTRACT

*Vicia* (Papilionaceae) has 45 species in Iran. Many biological activities have been reported from this genus. Several medicinal plants have accepted antihypoxic activities. Nothing is known about protective effects of *Vicia hirsuta* against hypoxia-induced lethality. In this study, the protective effects of *V. hirsute* aerial parts against hypoxia-induced lethality in mice were evaluated by three experimental models of hypoxia, asphyctic, haemic and circulatory. Statistically significant protective activities were established in some model of hypoxia in mice. Antihypoxic activity was especially pronounced in asphytic and haemic hypoxia. These effects were dose-dependent. Extracts at all tested doses, showed statistically significant activities respect to the control. In asphytic hypoxia, at 100 mg kg<sup>-1</sup>, extract significantly prolonged the latency for death with respect to control group (51.00 ± 6.05 vs. 33.67 ± 4.50 min, p<0.001). Phenytoin kept mice alive for 55.00 ± 6.05 min. In haemic model, control group died of hypoxia in 7.87 ± 0.78 min. Extract at 100 mg kg<sup>-1</sup> significantly prolonged latency for death with respect to control group (15.60 ± 1.34 min, p<0.001). In circulatory hypoxia, extract at 100 mg kg<sup>-1</sup>, significantly prolonged the latency for death with respect to control group (14.83 ± 0.75 vs. 10.71 ± 1.12 min, p<0.001). *Vicia hirsuta* showed a very good protective effect against the hypoxia in some model. The presence of polyphenols in this plant may be a proposal mechanism for reported antihypoxic activities of this plant.

**Keywords:** Antihypoxia, Asphyctic hypoxia, Phenytoin, *V. hirsute*.

### INTRODUCTION

The imbalance between low oxygen supply and oxygen demands determines organ hypoxia. It occurs especially in heart diseases, ischemia and heart attack, causing numerous deleterious effects and finally resulting in death (Kiang JG and Tsen K. 2006). Hypoxia causes oxidative stress involving production of reactive oxygen species (ROS) (Maiti P 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 ones. Among the various medicinal plants, some endemic and edible species are of particular interest because they

may be used for producing raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits (Exarchou V et al. 2002; Ebrahimzadeh, MA et al. 2009 and 2010a). *Vicia* genus member of Papilionaceae family has 45 species in Iran (Mozaffarian V 2006). The extract from *V. sativum* showed insecticidal activity. Also it has exhibited hepatoprotective effect against CCl<sub>4</sub> induced hepatotoxicity (Amarowicz R et al. 2008). Antioxidant activities of *V. faba* (Hashemi Z et al. 2014), *V. charca* and *V. sativa* (Orhan I et al. 2009) and *V. sativum* (Amarowicz R et al. 2008) have been reported previously. Also anti-inflammatory

and antinociceptive activity of *V. sativa* (Gamal-Eldeen AM et al. 2004) and antimicrobial and cytotoxic activity of *V. faba* (Akroum S et al. 2009) have been reported. There is no report on its biological activity *V. hirsuta*. The aim of this study was to determine the antihypoxic activities of *V. hirsuta* aerial parts against hypoxia-induced lethality in order to understand the usefulness of this plant.

## MATERIAL AND METHODS

### Animals

Male Swiss albino mice ( $20 \pm 2$  g) were randomly housed in groups of 10 in poly propylene cages at an ambient temperature,  $25 \pm 1^\circ\text{C}$  and 45-55% relative humidity, with a 12 h light: 12 h dark cycle (lights on at 7 a.m.). The animals had free access to standard pellet and water and *libitum*. Experiments were conducted between 8:00 and 14:00 h. 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.

### Asphyctic Hypoxia

The animals were subjected to hypoxia by putting them individually in a tightly closed 300 ml glass container which was placed under water in an aquarium of  $25^\circ\text{C}$ . The animals had convulsions and died from hypoxia. The latencies for death were recorded. The animals died approximately 2 min following convulsions. Mice received single *i.p.* injections of 10, 20 and 100 mg  $\text{kg}^{-1}$  doses of rutin or chlorogenic acid or phenytoin (50 mg  $\text{kg}^{-1}$ ) as 30 min before they were subjected to hypoxia. Another control group was treated with normal saline (Eslami B et al. 2001).

### Haemic Hypoxia

Forty mice were divided into five groups each containing eight mice. Control group was treated with normal saline. Thirty minutes after *i.p.* administration of 20 and 100 mg  $\text{kg}^{-1}$  doses of rutin or chlorogenic acid,  $\text{NaNO}_2$  (360 mg  $\text{kg}^{-1}$ ) was applied *i.p.* to mice and antihypoxic activity was estimated as the latent time of evidence of hypoxia in minutes (Ebrahimzadeh MA, et al. 2010b).

### 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 100 mg  $\text{kg}^{-1}$  doses of rutin or chlorogenic acid,  $\text{NaF}$  (150 mg  $\text{kg}^{-1}$ ) was applied *i.p.* to mice and antihypoxic activity was estimated in minutes as the latent time of evidence of hypoxia (Ebrahimzadeh MA, et al. 2010b).

### Statistical Analysis

Data were presented as mean  $\pm$  SD. 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 0.05 were regarded as significant.

## RESULTS AND 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 DS et al. 2004). Free radicals act as signaling species in various normal physiological processes but excessive production of these radicals causes damage to biological material (Bakony T and Radak Z. 2004). The increased level of ROS in hypoxia is the result of the accumulation of reduction equivalents in the mitochondrial electron transport system (Bakony T and Radak Z. 2004). The effects of ROS can be particularly evident in certain tissues such as brain because it consumes about 1/5 of the basal oxygen. 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. The survival time of animals in a sealed container directly reflects the anti-hypoxic activity. Oxygen deficiency of the brain leads to deleterious changes in structural and functional integrity of cerebral tissue. Consequently, any drug that enables the brain to resist the consequences of ischemia or hypoxia would be of great therapeutic interest (Peruche B et al. 1990). During the past decades a variety of different experimental models have been developed that could be used for testing antihypoxic and anti-ischemic drug effects *invivo* (Peruche B et al. 1990). Free radicals act as signaling species in various normal physiological processes but excessive production of these radicals causes damage to biological material. The increased

level of ROS in hypoxia is the result of the accumulation of reduction equivalents in the mitochondrial electron transport system (Bakony T and Radak Z. 2004). The effects of ROS can be particularly evident in certain tissues such as brain because it consumes about one fifth of the basal oxygen. 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 (Spencer JP. 2010). Statistically significant antihypoxic activities were established in some doses of *V. hirsuta* in experimental models of hypoxia in mice. The results of asphytic hypoxia are shown in Figure 1. The effects were dose-dependent. *V. hirsuta* at two tested doses showed statistically significant activity respect to the control. At 100 mg kg<sup>-1</sup>, it significantly prolonged the latency for death with respect to control group ( $51.00 \pm 6.05$  vs.  $33.67 \pm 4.50$  min,  $p < 0.001$ ). At 50 mg kg<sup>-1</sup>, it also prolonged survival time ( $38.75 \pm 4.06$  min,  $p > 0.05$  respect to control). Phenytoin that used as positive control kept mice alive for  $55.00 \pm 6.05$  min. This effect was statistically significant from the control ( $p < 0.001$ ). *V. hirsuta* at 100 mg kg<sup>-1</sup> showed the same activity of phenytoin ( $p > 0.05$ ). A close relationship between oxidative metabolism and cholinergic function has been found during the investigations of NaNO<sub>2</sub> on brain metabolism<sup>13</sup>. Chemical hypoxia is induced by the

injection of NaNO<sub>2</sub> (360 mg kg<sup>-1</sup>, i.p.), which reduces the oxygen-carrying capacity of the blood by converting hemoglobin to methemoglobin. This lethal dose is injected 30 min after the phenolic treatment. Immediately after the NaNO<sub>2</sub> injection, the animals are placed in small cages and the time between injection of NaNO<sub>2</sub> and cessation of respiration is recorded. Extract showed good activity in haemic model (Fig. 2). Control group died of hypoxia in  $7.87 \pm 0.78$  min. Extract at 100 mg kg<sup>-1</sup> significantly prolonged latency for death with respect to control group ( $15.60 \pm 1.34$  min,  $p < 0.001$ ). At 50 mg kg<sup>-1</sup>, it also prolonged the latency for death with respect to control group ( $9.64 \pm 1.03$  min,  $p < 0.05$  respect to control group). There are literature data that administration of NaF, that induces circulatory hypoxia, increases the blood histamine content and decreases the oxygen carrying capacity. The results of circulatory hypoxia are shown in Figure 3. Extract at 100 mg kg<sup>-1</sup>, significantly prolonged the latency for death with respect to control group ( $14.83 \pm 0.75$  vs.  $10.71 \pm 1.12$  min,  $p < 0.001$ ). This effect was also dose-dependent. At 50 mg kg<sup>-1</sup>, it also kept mice alive for  $13.80 \pm 1.26$  min. This effect was statistically significant from the control ( $p < 0.01$ ). The mechanism of this protective action may be due in part to the antioxidant activity of phenolic acids.

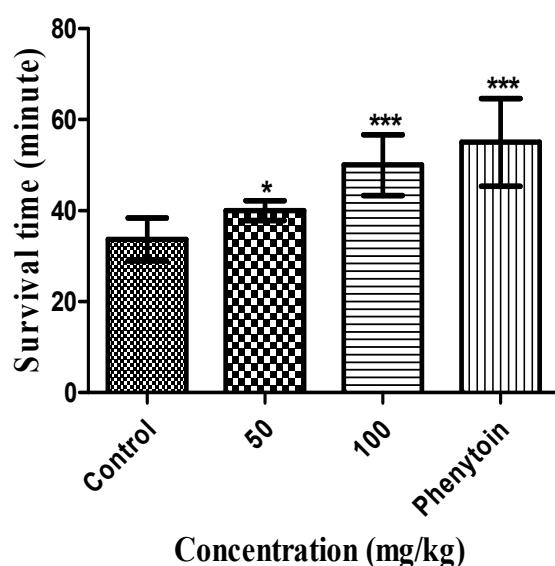
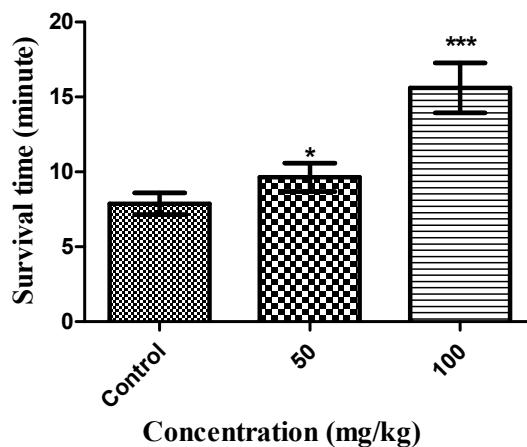


Figure 1

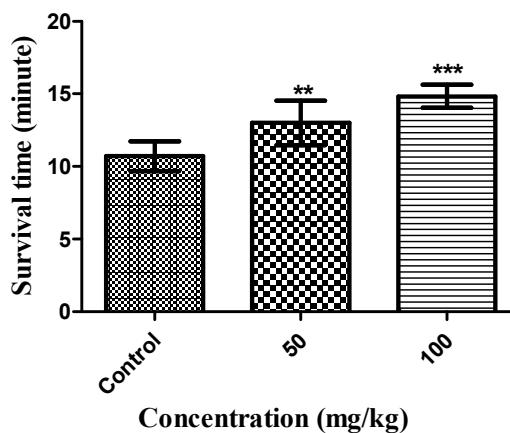
*Antihypoxic activities of V. hirsuta in asphyctic hypoxia in mice.*

*Data are expressed as mean  $\pm$  SD (n = 8), (\* $P < 0.05$ , \*\*\* $P < 0.001$ , compared to control).*

**Figure 2**

*Antihypoxic activities of *V. hirsuta* in haemic hypoxia in mice.*

*Data are expressed as mean  $\pm$  SD (n = 8), (\*P<0.05, \*\*\*P<0.001, compared to control).*

**Figure 3**

*Antihypoxic activities of *V. hirsuta* in circulatory hypoxia in mice.*

*Data are expressed as mean  $\pm$  SD (n = 8), (\*\*P<0.01, \*\*\*P<0.001, compared to control).*

## CONCLUSION

*Vicia hirsuta* showed a very good protective effect against the hypoxia in some model. Specifically, they produced significant and dose-dependent effect on the model of asphytic and haemic hypoxia. The presence of polyphenols in this plant

may be a proposal mechanism for reported antihypoxic activities of this plant.

## ACKNOWLEDGMENT

This research was supported by a grant from the Research Council of Mazandaran University of Medical Sciences.

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