

EFFECT OF METHANOLIC EXTRACT OF *HYOSCYMUS NIGER L.* ON THE SEIZURE INDUCED BY PICRITOXIN IN MICE

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ABSTRACT

Effects of *Hyoscyamus niger* L on central nervous system have been known for many years. The effects of methanolic extract of *H. niger* L. on seizures induced by picrotoxin was studied in mice in this investigation. In this study 7 groups of animals pretreated with methanolic extract of the plant (12.5, 25, 50, 100, 200, 300, 400 mg/kg/i.p.), 20 minutes prior to the picrotoxin (12 mg/kg/ i.p.) – induced seizures. Control mice received phenobarbital (40mg/kg/ i.p.) as positive control, or saline (10 ml/kg) as negative control. The latency of seizure (sec), duration of seizure (sec) and mortality rate were determined in test and control groups. The results of this study showed that latency of seizure was increased in groups that were pretreated with doses of 100, 200, 300 and 400 mg/kg of extract. In addition, methanolic extract of *H. niger* L. delayed the death time in mice as compared to control that was significant with doses of 200, 300 and 400 mg/kg. The most effective dose of extract was 300 mg/kg in this investigation ($P < 0.01$).

In conclusion, the results showed that methanolic extract of *H. niger* L. posses the anticonvulsant activity against picrotoxin-induced seizures in mice. The exact mechanism(s) by which the plant exerts its anticonvulsant activity is not determined yet.

Keywords: *Hyoscyamus niger* L., seizure, picrotoxin, mice.

INTRODUCTION

Hyoscyamus niger L is a plant growing in all parts of Iran (Zargari, 1990). The chemical characterization of plant shows the presence of different alkaloids such as hyocyamine, hyoscyne (scopolamine) and atropine which are proved to have anticholinergic (parasympatholytic) effects (Nicol, 2007).

In ancient Iranian medicine, the plant has been used for diarrhea, stomach pain and some central nervous system disorders such as parkinsonism, hysteric patients and seizures (Zargari, 1990).

There are controversial reports for anticonvulsant activity of anticholinergic drugs (Anderson *et al.*, 1994; Aucamp and Mayer, 1979; MCDonough *et al.*, 2000). Although anticholinergic drugs reported to be effective against nerve gas induced seizures (Anderson *et al.*, 1994; MCDonough *et al.*, 2000), however, Aucamp and Meyer, reported that epileptic seizures were exacerbated by anticholinergic drugs (Aucamp and Mayer, 1979). So the probable anticonvulsant effects of *H. niger* L is not proved yet and could be exerted by other chemical components of this plant or could be mediated through different mechanism(s). So we decided to test the probable anticonvulsant effects of methanolic extract of

H. niger L in an animal model of epilepsy. In this research, generalized seizure was induced by picrotoxin, a widely used as a model for chemically-induced convulsion in mice (Mackenzie *et al.*, 2002; Ngo Bum *et al.*, 2004; Buznego Perez-Saad, 2004; Heidari *et al.*, 2006b). This study is an attempt to establish a scientific basis for the use of this plant as antiepileptic in Iranian traditional medicine.

MATERIALS AND METHODS

Plant material

H. niger L was purchased from Zar Band Herbal Company, Tehran, Iran. The plant was authenticated by Botany Department of Bahonar University of Kerman and Pharmacognosy Department of Kerman Faculty of Pharmacy, as *H. niger* L (Family: Solanaceae). Voucher specimen of this plant has been deposited in Herbarium of Pharmacognosy, (No.1006) Kerman Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran (Zargari, 1990).

Preparation of Hyoscyamus niger methanolic extract

100 g of sliced, air dried seeds of *H. niger* L were milled into fine powder in a commercial blender. The powder was extracted with 80% aqueous methanol by percolation (72h) method (Samsam-Shariat, 1992). The methanolic

extract was concentrated to dryness under reduced pressure at $40\pm 1^\circ\text{C}$ in a rotary evaporator. The methanolic extract was freeze-dried, finally giving 8.02g (i.e., 8% yield). Aliquot portion of crude methanolic extract residue were weighed and dissolved in saline for final suitable concentrations for use on each day of our experiments (12.5, 25, 50, 100, 200, 300 and 400mg/ 10ml) (Heidari *et al.*, 2006b).

Animals

Adult male mice (Kerman Neuroscience Research Center), weighing 25-30g were used. The animals were housed in standard cages with free access to food (standard laboratory rodent's chow) and water (Heidari *et al.*, 2006a). The temperature of animal house was maintained at $22\pm 1^\circ\text{C}$ with a 12-h light/dark cycle (light on from 06:00 to 18:00 h). The ethical guidelines for the experimental seizures investigation in conscious animals were followed in all tests. All efforts were made to reduce the number of animals used and to minimize animal suffering (Zimmermann, 1983). Each animal was tested once.

Picrotoxin (PIC) test

Seizure were induced in male mice by injection of 12mg/kg PIC, i.p. (Ngo Bum *et al.*, 2004; Heidari *et al.*, 2006a). The general clonus was distinguished by clonus of all four limbs with transient loss of righting reflex (Mackenzie *et al.*, 2002; Buznego and Perez-Saad 2004; Heidari *et al.*, 2006a). This dose (12mg/kg) was administered to 9 groups of 10 mice 20 min after injection of either extract 12.5, 25, 50, 100, 200, 300 and 400mg/kg/i.p., Phenobarbital 40mg/kg, as positive control, saline 10ml/kg, as negative control (Heidari *et al.*, 2006a).

The onset time of seizure, latency (sec) (the time between the injection and the onset of first jerk or clonus), duration of seizure (sec), death latency (sec) and death rate was measured in the test and control groups (Heidari *et al.*, 2006a; Navarro Ruiz *et al.*, 1995; Avallone *et al.*, 2000; Swinyard 1969). The animals were observed for 90 min after picrotoxin injection.

Chemicals

Picrotoxin was purchased from Sigma (Poole, UK). Phenobarbital was purchased from Darupakhsh Company (Tehran, Iran). Methanol was purchased from (Merck, Germany).

STATISTICAL ANALYSIS

Results are presented as Mean \pm SEM and statistical significance between groups was analyzed by ANOVA followed by Newman-Keuls test. In all cases P values less than 0.05 were taken as statistical significance (Heidari *et al.*, 2006a,b; Navarro Ruiz *et al.*, 1995).

RESULTS

Effect of *H. niger* extract on the onset time of seizure induced by picrotoxin

Picrotoxin (12mg/kg) i.p.) produced generalized seizures in all the 10 control mice used. *H. niger* methanolic extract 100, 200 ($P<0.05$), and 300 mg/kg ($p<0.01$) produced significant delay in the onset time of seizures induced by picrotoxin (fig. 1). Lower doses of plant extract did not show any significant effect on the onset time of seizure induced by picrotoxin in mice (fig. 1). The reference anticonvulsant drug used, Phenobarbital (40mg/kg i.p.) also significantly delayed ($P<0.01$) the onset time of picrotoxin induced seizures (fig. 1).

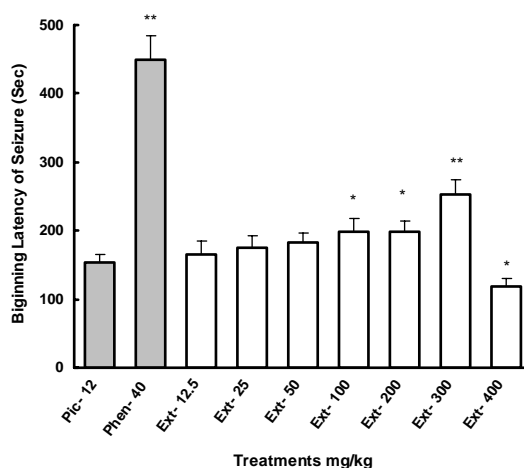


Fig. 1: The effect of *H. niger* extract on the onset time of seizure.

Normal saline 10 ml/kg, Phenobarbital (Ph) 40mg/kg or different doses of *H. niger* extract were injected intraperitoneally 20 minutes before picrotoxin 12mg/kg/ i.p. Data are the Mean \pm SEM. of 10 mice in each group.

*, $P<0.05$; significant difference from control group
**, $P<0.01$; significant difference from control group

Effect of *H. niger* extract on death latency

H. niger extract at the dose of 200 ($P<0.05$), 300 ($P<0.01$) and 400 ($P<0.05$) mg/kg i.p. significantly increased the death latency from seizure induced by picrotoxin. In other word, the time to death was increased from 1054 sec in picrotoxin treated mice to 1505 sec in mice received 300mg/kg *H. niger* extract (fig. 2).

Effect of *H. niger* extract on duration of seizure

H. niger extract increased the duration of seizures at doses of 200, 300 & 400mg/kg. However the severity of seizures was milder than control (fig. 3).

Effect of *H. niger* extract on death rate

H. niger extract had no effect on mortality rate from seizure induced by picrotoxin in this experiment, therefore the death rate was 100%.

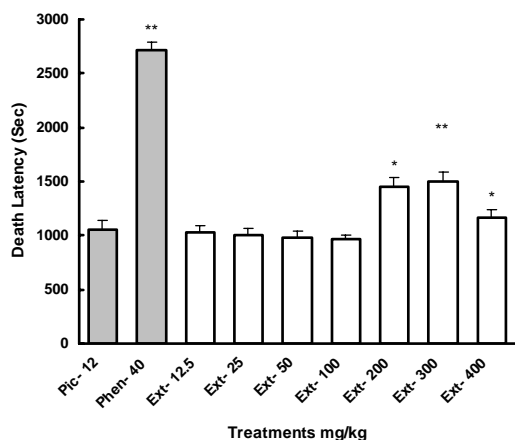


Fig. 2: The effect of *H. niger* extract on Death time induced by seizure.

Normal saline 10 ml/kg, Phenobarbital (Ph) 40mg/kg or different doses of *H. niger* extract were injected intraperitoneally 20 minutes before picrotoxin 12mg/kg. Data are the Mean \pm SEM. of 10 mice in each group.

*, P<0.05; significant difference from control group

** , P<0.01; significant difference from control group

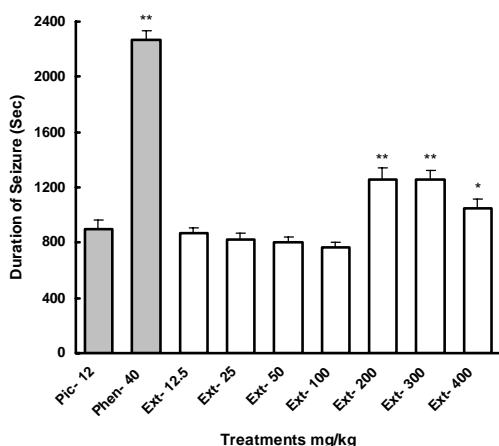


Fig. 3: The effect of *H. niger* extract on the duration of seizure.

Normal saline 10 ml/kg, Phenobarbital (Ph) 40mg/kg or different doses of *H. niger* extract were injected intraperitoneally 20 minutes before picrotoxin 12mg/kg. Data are the Mean \pm SEM. of 10 mice in each group.

*, P<0.05; significant difference from control group

** , P<0.01; significant difference from control group

DISCUSSION

The results of the present laboratory animal study provide evidence in favour of the anticonvulsant activity of the herb, and show that aqueous extract of *H. niger* possesses anticonvulsant activity in the experimental animal model used.

Picrotoxin (PIC), a potent, selective GABA-A receptor antagonist (Heidari *et al.*, 1996), produces seizures by blocking the effect of GABA at central GABA-A receptors, which have been associated with epilepsy (Nicol 2007 and Gale, 1992). Postsynaptic GABA-A receptors are functionally linked to benzodiazepine receptors, barbiturate receptors and chloride ion channels to form GABA-chloride ionophore complex, which is intimately involved in the modulation of GABAergic neurotransmission epilepsy (Nicol 2007; Gale 1992; Olsen 1981). According to Nicoll, picrotoxin, a GABA-A receptor antagonist, produces seizures by blocking the chloride-ion channels linked to GABA-A receptors, thus preventing the entry of chloride ions into the brain (Nicol, 2007). This process will, in turn, inhibit GABA neurotransmission and activity in the brain. Phenobarbital is believed to enhance GABAergic neurotransmission by increasing chloride ion flux through the chloride-ion channels at GABA-A receptor sites (Navarro Ruiz *et al.*, 1995; Olsen 1981). This hypothesis may explain the observed protective effects and/or antagonistic actions, of phenobarbital against PIC-induced seizures in the mice used.

However, *H. niger* methanolic extract only partially and weakly antagonized PIC- induced seizures, and was much less effective in this regard compared with Phenobarbital. This observation also tends to suggest that *H. niger* interferes with GABAergic neurotransmission, but probably not through GABA-A receptor sites (Mahomed and Ojewole 2006). *H. niger* have been reported to contain different alkaloids such as hyoscyamine, hyoscyne(scopolamine) and atropine which are proved to have anticholinergic (parasympatholytic) effects (Zargari 1990; Nicol 2007). Although anticholinergic drugs reported to be effective against nerve gas induced seizures (Anderson *et al.*, 1994), however, Aucamp and Meyer, reported that epileptic seizures were exacerbated by anticholinergic drugs (Aucamp and Meyer, 1979). So we are not certain about the potential ability of *H. niger* methanolic extract to prevent PIC-induced seizures through its anticholinergic mechanism.

However, our present state of knowledge of the chemical constituents of the extract is limited. It is, therefore, impossible for us at this stage, to pin-point and identify with certainty, the anticonvulsant principle's of the extract. Although the three major chemical constituents of *H. niger* that have been frequently reported in biomedical literature, namely: hyoscyamine, hyoscyne (scopolamine) and atropine (Zargari, 1990 and Nicol, 2007), are likely to account for the observed anticonvulsant activity of the plant's extract, there is no sufficient scientific data or evidence to back-up and justify this speculation. However, the experimental evidence obtained in the present laboratory animal study indicates that *H. niger* methanolic extract significantly delayed the onset of seizures induced by picrotoxin. Since picrotoxin-induced

seizures have been shown to be due to inhibition and/or attenuation of GABAergic neurotransmission, it is not unreasonable to speculate that *H. niger* methanolic extract probably produces its anticonvulsant activity by enhancing GABAergic neurotransmission. The observed anticonvulsant activity of the plant's extract may also be due, at least in part, to its ability to depress the central nervous system (CNS) by one or more of the known mechanisms of anticonvulsant action (McDonald, 1994). Chemical composition of *Hyoscyamus* may be changed by different condition of growth (Iftikhar et al., 1990). Three withanolide class steroids were isolated from the seeds of *Hyoscyamus niger*. Two of them were identified as daturalactone-4 and Nic-3 (which is now named hyoscyamilactol). The new compound was elucidated as 16 α -acetoxyhyoscyamilactol on the basis of spectroscopic properties and X-ray crystallographic analysis (Ma et al., 1999). Four lignanamides, a tyramine derivative, and 10 other nonalkaloidal components were isolated from the seeds of *Hyoscyamus niger*. Among them, hyoscyamide, 1,24-tetracosanediol diferulate, and 1-O-(9Z,12Z-octadecadienyl)-3-O-nonadecanoyl glycerol are new structures. The other compounds were identified as grossamide, cannabisin D, cannabisin G, N-trans-feruloyl tyramine, 1-O-octadecanoyl glycerol, 1-O-(9Z,12Z-octadecadienyl) glycerol, 1-O-(9Z,12Z-octadecadienyl)-2-O-(9Z,12Z-octadecadienyl) glycerol, 1-O-(9Z,12Z-octadecadienyl)-3-O-(9Z-octadecenyl) glycerol, rutin, vanillic acid, beta-sitosterol, and daucosterol (Ma et al., 2002).

Some reports showed anxiolytic, sedative, analgesic and anticonvulsant effects of some natural and synthetic flavonoids in animal experiments, and found that these compounds exerted their effects through the central benzodiazepine receptors (Salgueiro et al., 1997; Medina et al., 1997; Du et al., 2002; Heidari et al., 2005; Heidari et al., 2006b). Therefore, it seems that the antiseizure effect of *H. niger* may be related in part to flavonoid compound, rutin (Ma et al., 2002) present in the extract. However determination of the role of each compounds in the anticonvulsant effect of the extract is a wide field for more investigations

In conclusion, the findings of the present laboratory animal study weakly support the suggested anecdotal, folkloric, ethnomedical uses of *H. niger* in some forms of seizures. The exact mechanism(s) by which the *H. niger* exerts its anticonvulsant activity is not determined yet and needs further investigation to elucidate the other active compounds and underlying mechanism(s).

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