SHORT COMMUNICATION

INVESTIGATION OF HYPERICUM PERFORATUM EXTRACT ON CONVULSION INDUCED BY PICROTOXIN IN MICE

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ABSTRACT

Therapeutic effect of *Hypericum perforatum* L. has been well known. The aim of this study is to investigate the anticonvultant effects of *Hypericum* methanolic extract against seizure induced by picrotoxin in mice.

The study were performed on four groups of animals. They received percolated extract of *Hypericum perforatum* at the doses of 25, 50, 100 & 200 mg/kg intra peritoneally. After 20 minutes animals received picrotoxin 10 mg/kg for induction of seizure. Latency of seizure, duration of seizure, death latency and percent of mortality were determined.

The results indicated that latency of seizure increased in pretreated group with the dose of 50 mg/kg ($p<0.01$). The higher dose of extract 200 mg/kg significantly decrease duration of seizure and death latency. It maybe due to unknown ingredients in this plant or producing concentrations higher than the therapeutic level. The results showed that *Hypericum perforatum* L. at the dose of 50 mg/kg maybe have some beneficial effect in seizure induced by picrotoxin and this plant is suitable for continuing search in this field.

Keywords: *Hypericum perforatum* L., picrotoxin, seizure, mice.

INTRODUCTION

Epilepsy is a term used for major neurological disorder characterized by recurrent unprovoked seizures with an incidence of 0.5–1% in people worldwide. Modern drug therapy, even multi-drug therapy of epilepsy is not effective in some patients and associates with unwanted effects. Around 30% of these patients continue to have seizures with current AED (Anti epileptic drug) therapy (Smith and Bleck, 1991). An increasing number of patients and physicians in the developing countries use herbal medicines or folk remedies. In many cases traditional medicine are considered as a nontoxic substitute to synthetically manufactured drugs (Akerele, 1991). As many of the herbal drugs have few adverse effects, evaluation of herbal medications for their potential antiepileptic activity seems to be essential (Raza and Choudhary, 1999). *Hypericum perforatum* L., commonly named St. John's wort, is an herbaceous perennial plant of Hypericeae family, known for its putative medicinal properties including wound-healing, headache, diuretic , antibiotic paralysis, anti tetanus and anti spinal convulsion possessions (Mauri and Pietta, 2000 ;Öztürk *et al.*, 1996). Hypericum perforatum extracts contain a lot of constituents such as phenolic acids, a broad range of flavonoids, naphthodianthrones (hypericin and pseudohypericin) and phloroglucinols (hyperforin and adhyperforin) (Greeson *et al.*, 2001). The aim of this study is to evaluate the anticonvulsant activity of the methanolic extracts of *Hypericum perforatum* flowers against picrotoxin induced seizure on mice.

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MATERIALS AND METHODS

Plant

Hypericum perforatum L. purchased from Zarband Herbal Company, Tehran, Iran. The plant authenticated by botany deparment, Kerman university of Bahonar and Pharmacognosy Department, Kerman Faculty of Pharmacy, as *Hypericum perforatum*. Voucher specimen of this plant has been deposited in herbarium of pharmacognosy, (No.1008) Faculty of Pharmacy, Kerman University of Medical Sciences.

Preparation of methanolic extract

100 g of *Hypericum perforatum* L. flowers were cut into small pieces, homogenized using a blender and suspended with stirring in 80% methanol and macerated for 16 h at room temperature. The extract filtered (Samsam-Shariat, 1992), and then evaporated in a vacuum rotary evaporator

under low pressure at 40 ± 1˚C. The remaining extract was freeze-dried to obtain 8.02g (i.e. 8% yield) of dark-brown, powdery, crude methanolic extract. Extract residue were weighted and dissolved in saline for use in our pharmacological experiments (Heidari *et al.*, 2006b ;Mandegary *et al.*, 2004).

Animals

Male adult mice on 35-40 old-day, weighting 22-28g from Kerman Neuroscience Research Center were used. The animals were housed in standard cages at 23°C on a 12 h light-dark cycle. They were supplied with food and water ad libitum. The ethical guidelines for the experimental seizure investigations in conscious animals were followed in all tests (Zimmermann, 1983). Each animal was used once (Heidari *et al.*, 2007 ; Heidari *et al.*, 2006b).

Chemicals

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

Picrotoxin (PIC) test

Injection of 10 mg/kg PIC, i.p., induced seizure by acting as a non-competitive antagonist at GABAA receptor (Heidari *et al.*, 1996). 20 min after injection of either extract (25, 50, 100 & 200mg/kg, i.p.), phenobarbital (40mg/kg, i.p.) as positive control and saline (10ml/kg, i.p.) as negative control. Picrotoxin was administrated at the dose of 10mg/kg to all animals (Heidari *et al.*, 2009; Heidari *et al.*, 2006a). The mice were monitored for 90 min for 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, after picrotoxin injection (Avallone *et al.*, 2000; Mackenzie *et al.*, 2002).

STATISTICAL ANALYSIS

Data were expressed as mean± S.E.M. and were analyzed by one-way ANOVA followed by the Newman-Keuls test. P value less than 0.05 was the critical criterion for statistical significance (Heidari *et al.*, 2006a; Heidari *et al.*, 2007; Navarro Ruiz *et al.*, 1995).

RESULTS

Effect of Hypericum perforatum extract on the onset time of seizure induced by picrotoxin

Picrotoxin (10mg/kg, i.p.) produced generalized seizure in all groups. *H perforatum* L. methanolic extract at the dose of 50mg/kg produced significant delay ($p<0.01$) in the onset time of seizure induced by picrotoxin. Extract at the other doses did not show any significant effect on the onset time of seizure induced by picrotoxin. Phenobarbital as the reference anticonvulsant drug (40mg/kg i.p.) also delayed ($P<0.01$) the onset time of picrotoxin induced seizure significantly (fig. 1).

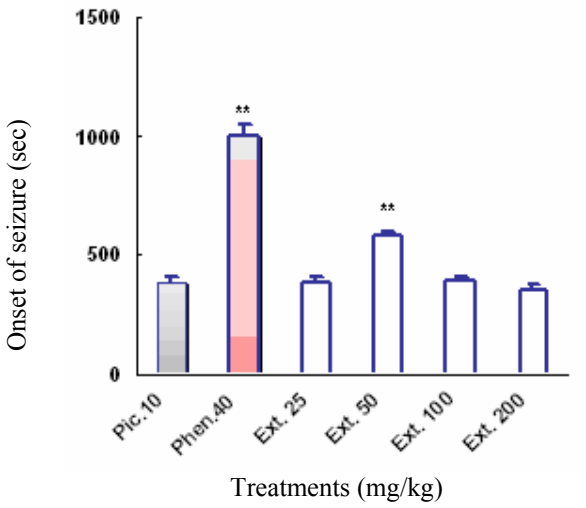

Fig. 1: The effect of *H. perforatum* extract on the onset time of seizure.

Normal saline 10 ml/kg, Phenobarbital (Phen) 40mg/kg or different doses of *H. perforatum* extract were injected intraperitoneally 20 minutes before picrotoxin 10mg/kg/ i.p. Data are the Mean ± SEM. of 7 mice in each group.

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

Effect on death latency

H. perforatum extract at the doses of 25, 50 and 100mg/kg, i.p. did not change the death time of seizure, while the dose of 200mg/kg decreased it significantly ($P<0.01$) (fig. 2).

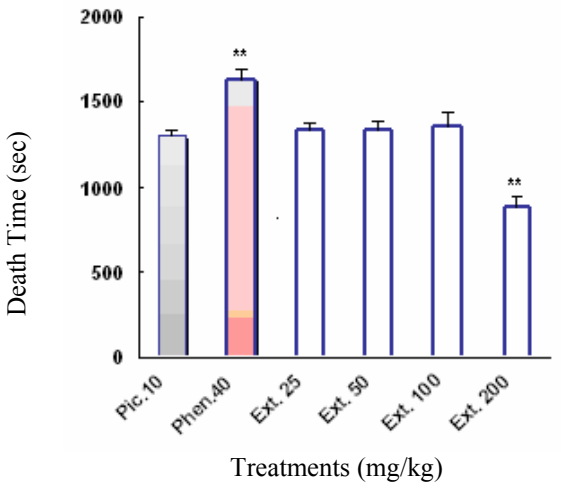

Fig. 2: The effect of *H. perforatum* extract on Death time induced by seizure.

Normal saline 10 ml/kg, Phenobarbital (Phen) 40mg/kg or different doses of *H. perforatum* extract were injected intraperitoneally 20 minutes before picrotoxin 10mg/kg. Data are the Mean ± SEM. of 7 mice in each group.

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

Effect on duration of seizure

The doses of 50 and 200mg/kg of extract decreased the duration of seizure significantly in comparison with the control group (P<0.05, P<0.01 respectively). However the severity of seizure was lower than control group (fig. 3).

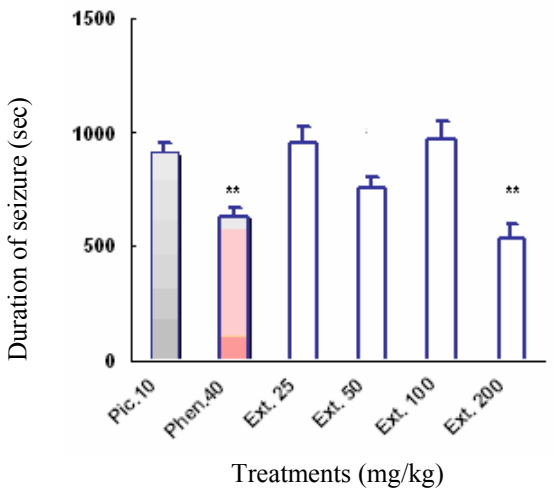

Fig. 3: The effect of *H. perforatum* extract on the duration of seizure.

Normal saline 10 ml/kg, Phenobarbital (Phen) 40mg/kg or different doses of *H. perforatum* extract were injected intraperitoneally 20 minutes before picrotoxin 10mg/kg. Data are the Mean ± SEM. of 7 mice in each group.

*P<0.05; significant difference from control group

**P<0.01; significant difference from control group

Effect on mortality rate

H. perforatum extract had no effect on mortality rate of seizure induced by picrotoxin, therefore the death rate was 100%.

DISCUSSION

The present study examined the anticonvulsant effect of *H. perforatum* using the picrotoxin -model.

Picrotoxin is a noncompetitive antagonist of γ-Aminobutyric acid -A receptors, the major inhibitory neurotransmitter receptor (Nicol, 2007). This compound produces seizure by prevention of ion flow through the chloride channel in the GABA-A receptor of nervous system (Gale, 1992; Nicol, 2007; Olsen, 1981). Phenobarbital increases the activity of GABA neurotransmitter by enhancing chloride ion flux through the chloride-ion channels at GABA-A receptor sites (Navarro Ruiz *et al.*, 1995; Olsen, 1981). This theory may elucidate the observed protective effects and/or antagonistic actions, of phenobarbital against picrotoxin -induced seizures in mice.

In this experiment, the dose of 50 mg/kg of *H. perforatum* extract delayed the onset time of seizure (P<0.01). The fact that the extract protected animal against picrotoxin induced seizure, suggest that it may contains compound(s) that facilitate GABAergic transmission. The ingredients of this plant include phenolic acids, a broad range of flavonoids, naphthodianthrones (hypericin and pseudohypericin) and phloroglucinols (hyperforin and adhyperforin) (Hosseinzadeh *et al.*, 2005). The anticonvulsant activities of this plant maybe related to its hyperforin, a major active constituent that inhibits the neuronal uptake of GABA (Wonnemann *et al.*, 2000). Also, an in vitro study has demonstrated that Hypericum extract displays affinity for 5-HT1, gammaaminobutyric acid (GABA)-A, GABA-B and benzodiazepine receptors in the micromolar (6-10 mol/l) range (Cott, 1997). In another study (Perfumi *et al.*, 2002) showed anxiolytic effect of *H. perforayum* extract in rat and found this plant exerted their effect through binding at gamma-aminobutyric acid (GABA)(A) and GABA(B) receptors, inhibit GABA reuptake and evoke GABA release from synaptosomes. A laboratory animal study indicated that some flavonoids, including hyperoside, quercitrin, isoquercitrin, and amentoflavone, may elicit a sedative effect that could involve both benzodiazepine and GABA receptor agonists (Baureithel *et al.*, 1997). Another report displayed conflicting results with regard to the effect of hypericin on monoamine uptake. According to one literature report, hypericin had inhibition effect on monoaine oxidase at very high (IC50=10–3 mol/l) concentrations, and in another study found that hypericin had no affinity for adrenergic, GABA or benzodiazepine receptors (Greeson *et al.*, 2001). However, it is possible that the anticonvulsant activity of this plant might be partially mediated by nitric oxide pathway (Hosseinzadeh *et al.*, 2005).

The highest dose (200mg/kg) exerted a less anticonvulsant effect. The extract at the higher doses may produce concentrations higher than the therapeutic level and produce non pharmacologic or toxic effects. These non therapeutic effects can be attributed to unknown ingredients in this plant. The inverted U-shaped dose response relationship is relatively common with complex herbal products and also reported by other (Heidari *et al.*, 2009). This finding confirmed with a 16 year-old girl presented to the emergency department with seizure and confusion for overdose of *H. perforatum* (Karalapillai and Bellomo, 2007).

Therefore, it seems that the antiseizure effect of *H. perforatum* may be related in part to flavonoid compound and hyperforin present in the extract. However more experiments are needed for determination of the anticonvulsant role of each compound in the extract.

In conclusion, present results can be support the suggested anecdotal, folkloric, ethnomedical uses of *H. perforatum* in some forms of seizures. The exact mechanism(s) by which the *H. perforatum* 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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