Research Journal of Medical Sciences 5 (5): 247-251, 2011
ISSN: 1815-9346

Study of *Matricaria recutita* and Vincristine Effects on PTZ-Induced Seizure Threshold in Mice

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Abstract: Studies have shown that chamomile contains significant amounts of free amino acids and flavonoids that have anti-inflammatory, anti-seizure and anti fever effects. Vincristine-based chemotherapy is the major treatment of Hodgkin's and non-Hodgkin's lymphoma, nephroblastoma and acute lymphoblastic leukemia. Studies showed that vincristine has neuropathic effects in humans and different animal models. In this study, anticonvulsant effects of hydroalcoholic extract of *Matricaria recutita* and convulsant effects of vincristine using the standard method of chemical seizure caused by Pentylenetetrazole (PTZ) in mice was investigated. For this propose first group, received normal saline, group II received *Matricaria recutita* hydroalcoholic extract (200 mg kg^{-1}) as intraperitoneal, vincristine group III received (10 μg/kg/day) intravenously for 10 days in the fourth group of *Matricaria recutita* hydroalcoholic extract plus vincristine was used and the subsequent seizure threshold was determined for each group. The results of this study showed that PTZ-induced seizure threshold in control mice was 35.52±0.87 mg kg^{-1} and chamomile hydroalcoholic extract significantly ($p<0.05$) increased the PTZ seizure threshold. Seizure threshold in the vincristine group significantly ($p<0.05$) decreased. Simultaneous uses of vincristine and *Matricaria recutita* extract caused to significantly increased seizure threshold ($p<0.05$) in vincristine taking group. The existence of several types of antioxidants and flavonoids in *Matricaria recutita* that have antioxidant effects in addition to anticonvulsant effects of this plant are set to reduce the neuropathic effects of vincristine. However, the anticonvulsant effect in human and material mechanisms involved need further investigations.

Key words: *Matricaria recutita*, pentylenetetrazole, vincristine, seizure, mice, Iran

INTRODUCTION

An epileptic seizure, occasionally referred to as a fit is defined as a transient symptom of abnormal excessive or synchronous neuronal activity in the brain. The signs and symptoms of seizures vary depending on the type. Seizures may cause involuntary changes in body movement or function, sensation, awareness or behavior. Seizures are often associated with a sudden and involuntary contraction of a group of muscles and loss of consciousness. However, a seizure can also be as subtle as a fleeting numbness of a part of the body, a brief or long term loss of memory, visual changes, sensing/discharging of an unpleasant odor, a strange epigastric sensation or a sensation of fear and total state of confusion. A seizure can last from a few seconds to status epilepticus, a continuous group of seizures that is often life-threatening without immediate intervention. Therefore, seizures are typically classified as motor, sensory, autonomic, emotional or cognitive. After the active portion of a seizure there is typically a period referred to as postictal before a normal level of consciousness returns (Coulter, 2001; Faingold, 2004; Lott and Mcauley, 2001). Epileptic drug therapy in most patients is based on experimental seizure classification because diversity causes seizure drugs are less specific for each of these effects. About 1% of people are born with epilepsy and approximately 10% of the population will experience a seizure. Although, by standard treatment in 80% of the seizure can be controlled nevertheless the millions of people have uncontrolled epilepsy (Engel, 2001). Despite the many advances in the field of medicine and pharmacy, patients and epileptic seizure disorders always have been challenges physicians and researchers. Today in the treatment of epilepsy combinations of the three mechanisms are: strengthening gabaergic inhibitory currents, typically reduction of glutamatergic drive current and balanced ionic currents, particularly sodium ions,

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calcium and chlorine. In some cases with recurrence, toxicity and side effects of drugs increased and the patient should have a long period of treatment to over (Gale, 1992; Roger and Brain, 2001).

Matricaria recutita (MR) also spelled camomile is an annual plant of the composite family Asteraceae. It usually grows near populated areas all over Europe and Mediterranean region. It is widely introduced in temperate North America and Australia. MR is used medicinally to treat sore stomach, irritable bowel syndrome, sedative, analgesic, strengthen the nervous system and as a sleep aid. It is also used as a mild laxative and is anti-inflammatory and anti-bactericidal. A 2006 review of the medical literature reported a number of beneficial effects for chamomile in *in vitro* and animal tests. Research with animals suggests antispasmodic, anxiolytic, anti-inflammatory and some antimutagenic and cholesterol-lowering effects for chamomile (Bisset and Wichtl, 2001; Hadjiakhoondi and Baligh, 2003; Heidari *et al*., 2002; McKay and Blumberg, 2006; Vahidi and Dashti, 2007; Viola *et al*., 1995; Zargari, 1997). The previous study proved antinociceptive effects of MR in neuropathic mice (Abad *et al*., 2011). Active ingredients include farnesene, chamazulene, flavonoids (including apigenin, quercetin, patuletin and luteolin) and coumarin (Bisset and Wichtl, 2001; McKay and Blumberg, 2006; Tyler *et al*., 1988). MR contains flavonoids including apigenin that can have anticonvulsant effects solidarities by gabaergic system, it could have Gamma-Aminobutyric Acid (GABA) like and by opening chlorine channels hyperpolarize neurons and increase seizure threshold (Viola *et al*., 1995).

Seizure caused by GABA receptor antagonist known as Pentylenetetrazole (PTZ) usually used in rodent seizure models due to its repeatability and providing the situation for comparison of different chemical compounds anticonvulsants effects under standard conditions. The substance causing the seizure, probably research through interaction with $GABA_A$ receptors and antagonized chloride ions flow caused by the GABA (Huang *et al*., 2001).

Vincristine is a vinca alkaloid from the catharanthus roseus (Madagascar periwinkle). Vincristine binds to tubulin dimers, inhibiting assembly of microtubule structures. Disruption of the microtubules arrests mitosis in metaphase. Therefore, the vinca alkaloids affect all rapidly dividing cell types including cancer cells but also those of intestinal epithelium and bone marrow. Vincristine is delivered via intravenous infusion for use in various types of chemotherapy regimens. Its main uses are in non-Hodgkin's lymphoma as part of the chemotherapy regimen, Hodgkin's lymphoma in acute lymphoblastic leukemia and in treatment for nephroblastoma. The main side-effects of vincristine are peripheral neuropathy, hyponatremia, constipation and hair loss. Peripheral neuropathy can be severe and hence a reason to avoid, reduce or stop the use of vincristine (Chauvenet *et al*., 2003; Gomber *et al*., 2010; Graf *et al*., 1996). The most important symptoms of neuropathic damage resulting from use of this drug-related is loss of motor neurons but the drug causes seizures, mental changes, increased production of excessive Anti-Diuretic Hormone (ADH) (Rosenthal and Kaufman, 1974). It seems that drug effects on the nervous system as an encephalopathy in human clinical cases including a seizure report in four patients noted that in spite of their use therapeutic dose of vincristine (1.5-2 mg/body m^3 once a week) (Johnson *et al*., 1973). In another case relating to the treatment of a patient with Hodgkin's lymphomas by vincristine, regional face seizure was observed that ended after 10 min (Dallera *et al*., 1984). This study was preformed because one of vincristine-induced neuropathic mechanisms is oxidative stress and *Matricaria recutita* contains different substances including antioxidants and flavonoids that could be useful in seizure. This study investigated the convulsant effects of vincristine and anticonvulsant effects of *Matricaria recutita* in mice.

MATERIALS AND METHODS

Animals: Experiments were performed on 25-30 g adult male NMRI male mice in their 8-9 weeks, purchased from Razi institute. Animals were acclimated to the laboratory environment for 5-7 days before being used in the study. Animals were housed 6 per cage in a temperature and humidity controlled environment under a 12 h light/dark cycle (lights on at 7 AM). Food and water were available *ad libitum*. The National Institutes of Health guidelines for care and use of animals and Guidelines on Ethical Standards Experiments in Animals were followed (Zimmerman, 1983). All efforts were made to minimize the number of animals which were used and their suffering degree.

The vincristine-induced neuropathy model was conducted by Intravenal (IV) injection in this experiment. Animals subsequently received daily IV injections of either vincristine sulfate (100 μg/kg/day), saline (0.1 mL/kg/day) MR hydroalcoholic extract (200 mg/kg/IP) for 12 days, immediately following PTZ-seizure testing. The treatment paradigm consisted of five daily injections followed by a 2 days interval where no injections were administered followed by five subsequent daily injections as described previously (Weng *et al*., 2003).

Animals were divided into 4 groups randomly, the first group received saline normal (control group), the second group received MR hydroalcoholic extract (200 mg/kg/IP) (MR group) (Heidari *et al*., 2009), the third

group received vincristine (100 μg/kg/IV/day) (Vin group) (Bujalska and Gumulka, 2008), the forth group received MR hydroalcoholic extract and vincristine (Vin+MR group).

Chemicals: PTZ were purchased from Sigma-Aldrich company and dissolved in normal saline. Vincristine was purchased from Tocris Cookson Ltd., Bristol, Avon, UK.

Administration of test agent: Vincristine (100 μg kg^{-1}) was administered intravenously via tail vein. Normal saline and MR hydroalcoholic extract was administered intraperitonealy. Dose selection of each agent was based on the results of previous studies (Bujalska and Gumulka, 2008; Heidari *et al*., 2009; Weng *et al*., 2003). To determine seizure threshold, PTZ solution (5 mg mL^{-1}) was infused in a constant rate of 0.5 mL min^{-1} into the lateral tail veins of mice. Infusion continued until the occurrence of upper limb clonic seizure and followed by full body tonic seizure. Minimum dose of PTZ (mg kg^{-1} of mice body weight) needed to create clonic seizure as an index of clonic seizure threshold was considered (Gholipour *et al*., 2009; Homayoun *et al*., 2002).

Extracting method: Dry MR flowers (*Matricaria recutita*) from Esfahan pharmaceutical company purchased and drench method used for extraction. For this purpose flowers mildly powdered. The 20 g of MR powder and 200 mL of 70% ethylic alcohol mixed and after 48 h (container were motivated for 5 min with 12 h withdrawal time). The mixture leached and solvent extracted in rotary adjusted in 70°C in medium round speed. The caliginous fluid was spread on a window and in 50°C oven and after drying the powder gathered and used in this experiment (Arzi *et al*., 2004; Bisset and Wichtl, 2001).

Data analysis: Group data are presented as mean±SEM and analyzed statistically using one-way ANOVA. The level for statistical significance was set at a p-value of <0.05.

RESULTS AND DISCUSSION

Seizure threshold obtained in mice that received normal saline as control was 35.52±0.87 mg kg^{-1}. Vehicles effect on seizure threshold showed that the vehicles used in this study didn't have significant effect on seizure threshold.

Intravenous administration of vincristine caused decrease in PTZ-induced seizure threshold to 23.6±1.04. This decrease was statistically significant ($p<0.05$) compared with control group (Fig. 1).

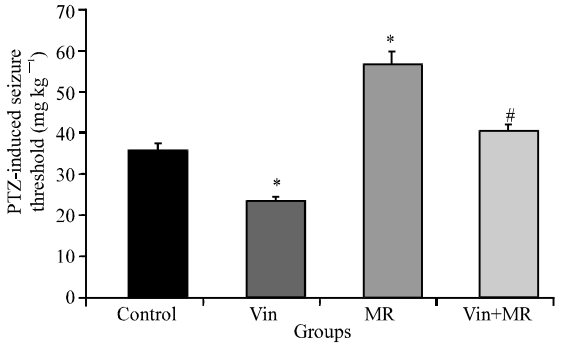

Fig. 1: Effect of hydroalcoholic extract of *Matricaria recutita*, Vincristine and *Matricaria recutita*-vincristine together on PTZ-induced clonic seizure threshold in mice. Each line represents mean±SEM of 6 mice; *$p<0.05$, vs. control group and # $p<0.05$, vs. vin group

Intra peritoneal injection of MR, 30 min before seizure test caused increase in threshold to 56.96±2.95. This increase was statistically significant ($p<0.05$) compared with control group (Fig. 1).

Injection of MR and vincristine together (Vin+MR group) before PTZ-induced seizure threshold test have shown that seizure threshold was 40.74±1.27. This increase was statistically significant ($p<0.05$) compared with vincristine group (Fig. 1).

Medical and therapeutic effects of traditional medicine and medicinal plants and harmless effects in many years is the major reason of using this kind of therapeutics yet. In this study the effect of MR hydroalcoholic extract on PTZ-induced seizure threshold was determined. $GABA_A$ receptor is prominent inhibitory neurotransmitter receptors in vertebrate central nervous system. When this receptor activated, receptor's chloride channels open, leading to flow of chloride ions and nervous hyperpolarization (Huang *et al*., 2001). The receptor has a multiple connection positions through which different drugs can adjustment GABA by chloride ions. Benzodiazepines and barbiturates are known as current amplifiers of GABA-induced chloride ions (Hevers and Luddens, 1998). Versus, drugs such as picrotoxin and several other drugs are known to suppress the chloride flow that mediated by GABA. It is well marked that PTZ acts on the position of picrotoxin action complex on GABA receptor (Huang *et al*., 2001). The advantages of this standard method is that due to high repeatability capability and provide the underlying model to compare the anticonvulsant nature of chemicals, PTZ are used to induce seizure in animal models.

In this study, MR increased PTZ-induced seizure threshold. Since PTZ acts via $GABA_A$ receptor, it seems that an anticonvulsant effect of MR is through Gabaergic

system. Other researchers showed with biochemical studies that MR extract containing apigenin that have GABA like effects (Viola *et al*., 1995) and it can increase GABA effects on $GABA_A$ receptors. Because GABA is an inhibitory neurotransmitter in brain it decreases activity in central nervous system (Barnes *et al*., 2002). Another mechanism proposed to have effects on central nervous system is interacting with body's histaminergic system (Miller *et al*., 1996).

Studies have shown that the anticonvulsant effects of MR extract is dose dependent (Arzi *et al*., 2004; Heidari *et al*., 2009). Arzi *et al*. (2004) have shown that MR could be useful on nicotine induced seizures. So, MR extract could have effects on glycine transport (inhibitory neurotransmitter in the spinal cord) (Arzi *et al*., 2004).

In present study the convulsant effects of vincristine evaluated as cancer chemotherapy agent. Although, the mechanism of vincristine as anticancer agent is an anti metabolites that inhibited cell proliferation in every stages (Rosenberg, 1977) but the most limiting factor is neuropathy mechanism is not well understood (Gomber *et al*., 2010). Aley *et al*. (1996) have shown that using vincristine in laboratory animals can be a model for neuropathic effects in humans. They have shown that administration of vincristine for 2 weeks in laboratory animals can induce neuropathic effects (Aley *et al*., 1996). It seems that drug effects on the nervous system as an encephalopathy in human clinical cases including a seizure report in four patients noted that in spite of their use therapeutic dose of vincristine. One of the mechanisms suggested for causing encephalopathy was neurons myelin peroxidation (Johnson *et al*., 1973). In one of the human cases treated with Vincristine therapeutic dose for 4 days general seizure was observed, in addition physicians treating her seizures with intermittent anticonvulsant drugs, nonsteroidal anti-inflammatory drugs and dexamethasone was used to control the symptoms (Scheithauer *et al*., 1985). To reduce vincristine side effects complementary therapies can be used including use of medicinal plants. Due to daily administration of MR extract in this study, it can be expected antioxidant effects of this plant overcome to side effects of this drug. Also because of substances that are effective in central nervous system, it seems generally the plant is useful to reduce convulsant symptoms caused by vincristine.

CONCLUSION

The results of the study showed that MR hydroalcoholic extract increased seizure PTZ-induced threshold in vincristine received mice. Probably gabaergic inhibitory neurotransmitter system is responsible for the anticonvulsant effect. However, the role of anticonvulsant mechanisms in human is needed further investigation.

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