Study of *Matricaria recutita* and Vincrietine
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. Vincreistine-based chemotherapy is the major treatment of Hodgkin’s and non-Hodgkin’s lymphoma, nephroblastoma and acute lymphoblastic leukemia. Studies showed that vincreistine has neuropathic effects in humans and different animal models. In this study, anticonvulsant effects of hydroalcoholic extract of *Matricaria recutita* and convulsant effects of vincreistine 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, vincreistine group III received (10 μg/kg/day) intravenously for 10 days in the fourth group of *Matricaria recutita* hydroalcoholic extract plus vincreistine 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 vincreistine group significantly (p<0.05) decreased. Simultaneous uses of vincreistine and *Matricaria recutita* extract caused to significantly increased seizure threshold (p<0.05) in vincreistine 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 vincreistine. However, the anticonvulsant effect in human and material mechanisms involved need further investigations.

**Key words**: *Matricaria recutita*, pentylenetetrazole, vincreistine, 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 epileptics, 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 gabaaergic 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; Hadjiaakooordi and Baligh, 2003; Heidari et al., 2002; McKay and Blumberg, 2006; Vahidi and Dashi, 2007; Viola et al., 1995; Zagari, 1997). The previous study proved anticonvulsive 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 solidaries by gababergic system, it could have Gamma-Aminobutyric Acid (GABA) like and by opening chloride channels hyperpolarize neurons and increase seizure threshold (Viola et al., 1995).

Seizure caused by GABA receptor antagonist known as Pentylentetrazole (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 Intravenous (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) (Buyalska 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 (Buyalska 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 dry method used for extraction. For this purpose flowers mildly powdered. The 20 g of MR powder and 200 mL of 70% ethyl 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. Vehiculs 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](image.png)

*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<sub>A</sub> receptor is prominent inhibitory neurotransmitter receptors in vertebrate central nervous system. When this receptor activated, receptor’s chloride channels open, leading to flowing 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 picrotxin 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<sub>A</sub> 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 \textsubscript{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; Heidar 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 convulsive effects of vincristine evaluated as cancer chemotherapy agent. Although, the mechanism of vincristine as antineoplastic agent is an anti metabolic 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 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 anticonvulsant 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 gabærgic inhibitory neurotransmitter system is responsible for the anticonvulsant effect. However, the role of anticonvulsant mechanisms in human is needed further investigation.

**REFERENCES**


