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Research Article

ANALYSING THE EFFECT OF HYDRO ALCOHOLIC EXTRACT OF *CICHORIUM INTYBUS* L. ON SEIZURES, EXPERIMENTALLY INDUCED BY PENTYLENETETRAZOLE IN SMALL MALE LAB MICE

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ABSTRACT
Epilepsy is the most common neural damage caused by brain strokes. Even with the variety of currently available antiepileptic drugs, the research to find newer, more efficient and less destructive medications is pursued. The current study was done to analyse the effect of *Cichorium intybus* hydroalcoholic extract on the seizure threshold caused by administration of pentylenetetrazole in small male mice. In this study Syrian NMRI mice (30-35gr) were divided into groups: a control group (0.55cc saline) and groups receiving various dosages of *Cichorium intybus* hydro alcoholic extract (15, 25, 50 & 100 mg/Kg). 30 minutes after administering the appointed dosages of the aforementioned extract, every mice was injected with 80 mg/Kg of PTZ in order to induce seizures. All injections were performed intraperitoneally. For a period of 30 minutes subsequent to PTZ administration, seizure threshold and the duration of seizure phases were recorded. The Toki test and one way ANOVA analysis were employed in data processing. Analysis indicated that when compared to the control group (receiving a solvent of the extract), receiving a dose of 25 mg/g of *Cichorium intybus* significantly increases seizure threshold as well as reducing the duration of each of the phases of epilepsy ($P<0.001$). The hydro alcoholic extract of *Cichorium intybus* has antiepileptic attributes.

Keywords: *Cichorium intybus Hydroalcoholic Extract, Pentylenetetrazole, Seizure (Epilepsy), Male Syrian Mouse*

INTRODUCTION
Epilepsy is a neural disorder, occurring when a sudden imbalance takes place between excitatory and inhibitory signals in a neural circuit, leading to a series of unnatural discharges. These events usually begin in a specific location which spread out to further regions in the cephalic cortex (Heyadri *et al.*, 1925; Zapata-Sudo *et al.*, 2010). Seizures are commonly a sign of epilepsy; however, the first attack does not confirm the presence of this condition. In addition, the patient's consciousness levels are usually affected by alternating, spontaneous, temporary and recurring attacks.
According to epidemiologic studies, more than 50 to 60 million of the current human population suffer from epilepsy (Arzi and Golahdar, 1972; Hafezieh, 1975; Kaplan and Rafi, 1973; Lucindo *et al.*, 2010; Sun *et al.*, 2010). Many factors have been defined as causative parameters in epilepsy; however the mechanism which each of such factors enforce is specific to itself, hence their variation. Different neurotransmitters, significantly GABA (Gamma Amino Butyric Acid) and Glutamate, play important roles in the pathogenesis of epilepsy (Holtman *et al.*, 2009). In spite of the major research performed in the field of antiepileptic drugs, this disease has remained incurable (Lucindo *et al.*, 2010). In addition, more than 50% of the patients who receive antiepileptic medication experience life threatening side effects. It is so that the search for more efficient and targeted medications still continues. GABA, the paramount inhibitory neurotransmitter of the brain, increases the flow of chloride ions into neurons, causing their membrane to hyperpolarize which in turn, inhibits the cell. Many neurons of the cerebral cortex are found to produce GABA, playing an important role in controlling epilepsy. Many experimental studies confirm the role of GABA in relation with seizures. Glutamate is considered the most significant excitatory neurotransmitter of the CNS, which activates the NMDA receptors. The antagonist of the

mentioned receptor has antiepileptic properties since a portion of the attacks happen due to electric discharge of the neurons or excess amounts of Calcium ions entering the cells. In short, many mechanisms may be responsible for the occurrence of seizures, the most common of which are:

1. Failure of inhibitory mechanisms especially GABA mediated synaptic inhibition.
2. Increased activity in excitatory mechanisms, specifically Glutamate and NMDA receptors.
3. Amplification of spontaneous self-excitation due to increased calcium flow.

Medicinal plants have been used to control and cure diseases since many centuries ago. The medicinal attributes of such plants, as well as their fewer and less dangerous side effects have been proven throughout the years of experimental and scientific practices.

Chicory, which carries the scientific name of *Cichorium intybus* L., is an important member of the Asteraceae family, belonging to the sub family of cichorieae and the tribe Lactuceae (Ghahreman, 1995). The chemical composure of Chicory consists of water, carbohydrates (Cellulose, amylum, sugars and inulin), proteins, poly acetylenes, lipids, minerals and a variety of tripnoids, especially sesquiterpenes and lactones (Majd and Sharif, 2007).

The pharmacologic attributes of this herb are as follows: anti-malaria (Bischoff *et al.*, 2004), anti-diabetic (Pushparaj *et al.*, 2007), antimicrobial (Hyung and Hyework, 1999), anti-hepatotoxicity (Ahmed *et al.*, 2003) and anti-allergic (Gazzain *et al.*, 2000) specificities, anti-inflammatory (Hassan, 2008) laxative (Sugatania *et al.*, 2003), anti-testicular toxicity (Sugatania *et al.*, 2003), diuretic (Pool-Zobel *et al.*, 2002; Kaur and Gupta, 2002), anti-neoplastic (Nayeemunnisa, 2009) attributes as well as neural protection (Marteau *et al.*, 2011), protection against the progress of acute pancreatitis (Tousch *et al.*, 2008). The administration of Chicory also lowers blood sugar levels (Abed). In accordance with the mentioned attributes, the main aim of this study is to analyse the effect of *Cichorium intybus* L. hydro alcoholic extract on PTZ induced seizures in small male lab mice.

MATERIALS AND METHODS

Chicory (roots, stem and leaves) were obtained from Mazandaran and identified and coded by the herbarium of Qom Islamic Azad University. Gathered specimen was dried in a temperature of 25°C and away from direct sunlight. Various parts of the chicory plant were processed and prepared for essence extraction. 40 grams of processed dried chicory was placed within a Coxhile balloon wrapped in a white cloth parchment, to which 300 cc of 70% ethanol was later added. A condenser was placed above the Coxhile balloon. This system was attached to a stand and placed over a flame. After 24 hours, the gathered extract was filtered. In order to evaporate the primary solvent, the extract was placed in an oven equipped with a fan in 38 degrees Celsius. The final product was powdered and transferred in small dark vials which were stored in a refrigerator.

Animals

Male Syrian NMRI mice weighing 30-35 gr and an age of 5-6 weeks were selected as study subjects. The mice were kept in a temperature of 18-22 degrees with a humidity level of 18-22%. All subjects had free access to water and special food. Light was automatically controlled in two 12 hours phases of darkness and light.

Study Groups

The control group received 0.55cc of normal saline, while the test subjects received 15, 25, 50 and 100 mg/kg doses of chicory hydro alcoholic extract by the way of Intraperitoneally injections. PTZ, obtained from Sigma co., was injected intraperitoneally in 80 mg/kg doses, 30 minutes after chicory extract administration. The duration of seizure phases were recorded directly after PTZ administration.

The phases of epilepsy are as follows:

Phase zero: no reaction (normal behaviour)

Phase one: twitching of the muscles of the face and ears as well as writhing of the frontal limbs (cranial myoclonic contractions)

Phase two: shock-like spams movements in the muscles (repeated cranial seizure accompanied by jerking spasms in frontal limbs)

Phase three: shock like spasms in the muscles accompanied by jumping and jerking movements (total body muscle contraction accompanied with jerking of the frontal limbs; feet are usually placed wide apart and the tail becomes stiff)
Phase 4: general colonic-tonic contractions throughout the body; subjects fall on one side or stand on two feet in some occasions.
Phase five: subject collapses in a supine position. General clinic- tonic contractions are present.
Analytical processing:
The data were analysed using ANOVA and Tukey tests (P<0.05).

RESULTS AND DISCUSSION

Results

- Results indicate that in comparison to the control group (0.5cc saline), when administered in 25 mg/Kg doses, hydroalcoholic chicory extract increases seizure threshold significantly (P<0.01)

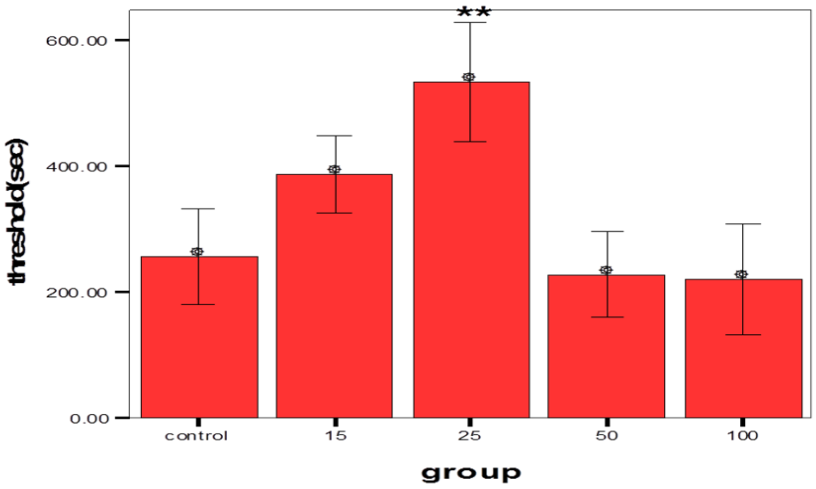

Figure 1: The effect of varying doses of hydroalcoholic chicory extract on seizure threshold

- Reports show that administration of hydroalcoholic chicory extract in 15, 50 and 100 mg/Kg doses significantly affects the duration of the first seizure phase. Compared to the control, subjects receiving a dose of 15 mg/Kg of the extract, the duration shows a meaningful increase while administering a dose of 25 mg/Kg reduces the duration of this phase (P<0.01)

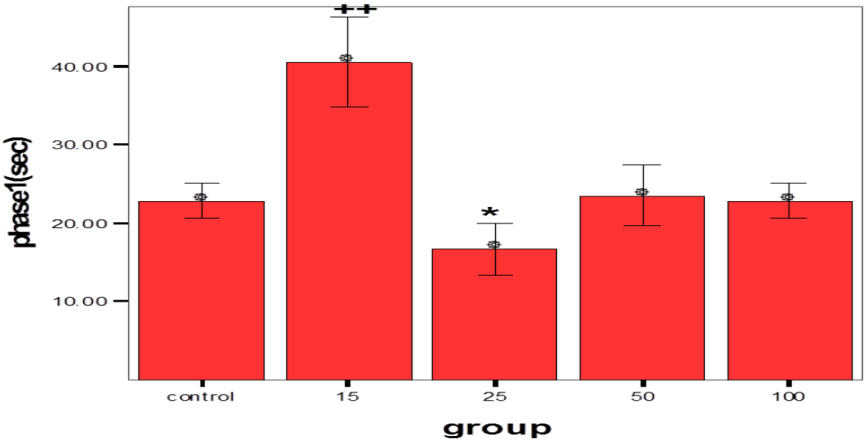

Figure 2: The effect of administering hydroalcoholic chicory extract on the duration of the first epileptic phase

- Results also show that between the group which received a dosage of 15, 25, 50 and 100 mg/Kg of hydroalcoholic chicory extract and the control, the duration of the third epileptic phase varies significantly. This duration had reduced significantly in the group which had received 25 mg/Kg of this extract, while in other groups which had received 15, 50 or 100 mg/Kg of the aforementioned extract, the duration of this phase showed no apparent change (P<0.01).

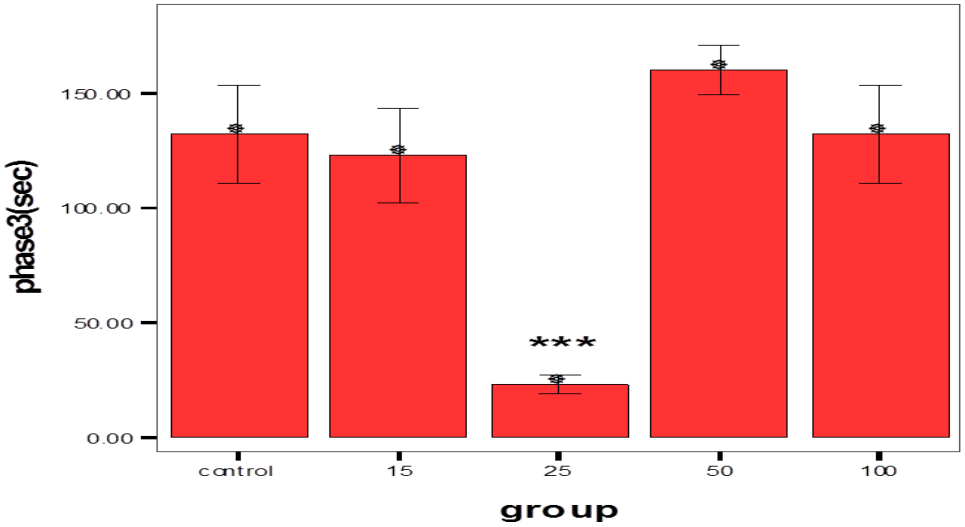

Figure 3: The effect of administering hydroalcoholic chicory extract on the duration of the third epileptic phase

- Results show that between the duration of the fourth epileptic phase (collapsing on one side accompanied by general colonic- tonic contractions) shows no significant variation between the control group and the group receiving hydroalcoholic chicory extract in either of the 15, 25, 50 and 100 mg/Kg dosages. When compared to control group, the duration of this phase shows a meaningful reduction in the group which had received 25mg/KG of this extract (P<0.01).

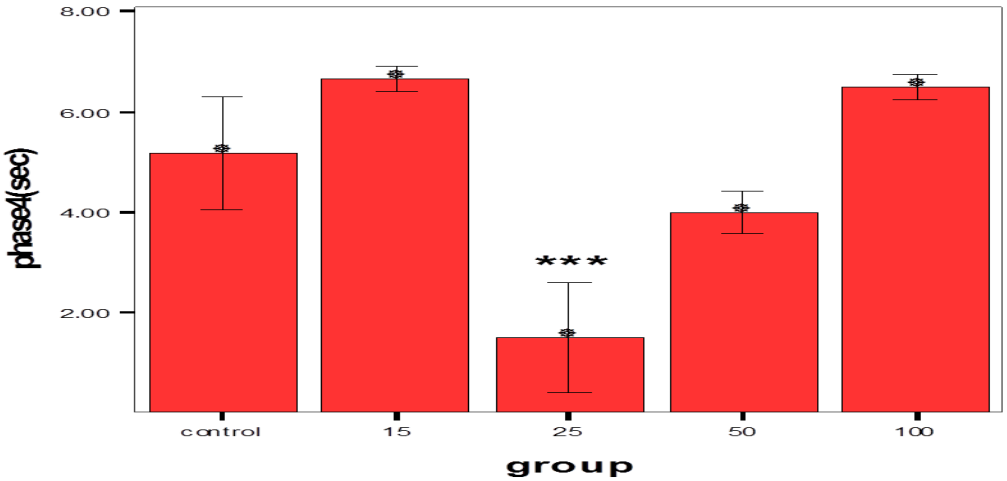

Figure 4: The effect of administering hydroalcoholic chicory extract on the duration of the fourth epileptic phase

- Results indicate that the duration of the fifth epileptic phase (characterized with supine collapsing and general colonic-tonic contractions) shows a meaningful variation between the control and the group

receiving either of the dosages of 15, 25, 50, 100 mg/Kg of hydro alcoholic chicory extract. The duration of this phase had reduced significantly in the first two test groups (15 & 25 mg/kg) (P<0.01)

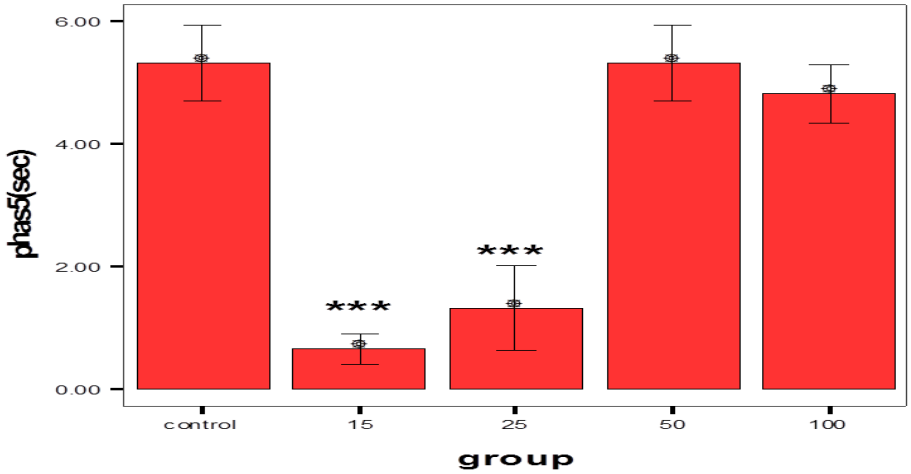

Figure 5: The effect of administering hydroalcoholic chicory extract on the duration of the fifth epileptic phase

Thirty minutes prior to administration of TPZ (80 mg/kg) male Syrian mice were injected intraperitoneally with various dosages (15, 25, 50 and100 mg/Kg) of a hydroalcoholic chicory extract. Subsequent to injection, the exact time in which the seizures started (seizure threshold) as well as the duration of each of the 5 epileptic phases was recorded. The presented figures show the results in the form of mean± standard deviation.

P<0.05
P<0.01
P<0.001

Discussion

Despite all the progress in the field of antiepileptic treatment, epilepsy remains an incurable disease. Thus, the search for medications which are more efficient compared to available chemical drugs still continues (Sugatania *et al.,* 2003). Recently, treatments based in natural resources and medicinal plants are receiving growing interest due to their efficiency and fewer side effects (Sugatania *et al.,* 2003). In this study, the effect of an hydroalcoholic chicory extract in 15, 25, 50 and 100 mg/Kg doses was analysed on an TPZ induced epilepsy in male Syrian mice. The gathered results indicate that when administered at a dosage of 25 mg/Kg, this hydroalcoholic extract of *Cichorium intybus* L. increases seizure threshold as well as reducing the duration of any of the 5 epileptic phases. In general, this extract has antiepileptic attributes. In recent studied performed by Olivera *et al.,* (2008), the destructive effect of Cyclooxigenase-2 on cephalic neurons was analysed; this enzyme may play a great role in the pathophysiology of diseases such as Alzheimer's and Parkinson's as well as in epilepsy (Olivera *et al.,* 2008; Olivera, 2008). One of the main components of this extract is tripnoids. In a study performed by Suh *et al.,* (1998), it was determined that tripnoids are capable of inhibiting Cox-2 production. Cox-2 catalysis the conversion of Arachidonic acid to prostaglandins. Also, according to the studies performed by Garavand *et al.,* (2010), cox-2 inhibitors such as refocoxib, Aspirin, ketoprofen and phallotoxin, all have antiepileptic attributes. In accordance with the importance of Cox-2 in epilepsy, it has been suggested that in increasing tonicity, this enzyme is a Glutamatergic compound while in decreasing tonicity, Cox-2 shows GABA-ergic attributes (Garavand *et al.,* 2010). It appears that epileptic seizures may be controllable by reducing glutamate secretion through cox-2 through inhibition; cox-2 itself is inhibited by the inhibition of E2 prostaglandins. In addition, the activation of Cox-2 leads to the production of free radicals which in turn increases oxidative stress and induces apoptosis in GABA-ergic

neurons. Thus, the concentration of Glutamate increases due to the removal of inhibitory effects of GABA neurons on Glutamate-ergic neurons which increases the activity of the latter cells, eventually resulting in amplified seizures. The inhibitory effects of GABA are pharmacologically restrained by administering bicuculline and picrotoxin, which inevitably ends in seizures (Garavand *et al.,* 2010). The receptors for Flavonoid attachment on the neurons are as follows: Adenosine, GABA and the receptor for testosterone. It is also assumed that benzodiazepine receptors are also affected. Currently, the structure of about 5000 herbaceous flavonoids has been explained. Many flavonoids act as ligands for GABA receptors throughout the CNS and thus, might have similar effect to benzodiazepine. In addition, Flavonoids are capable of crossing the blood-brain barrier and act as a positive allosteric regulator, amplifying the effect of GABA on their specific receptors (Garavand *et al.,* 2010).

In 1985, Alcaraz explained the mechanism of which Flavonoids use to close Calcium canals; he believes that flavonoids inhibit the activity of N-methyl D- Aspartate, thus reduce the calcium concentration within the cell (Alcaraz and Hoult, 1985). Glycosides are other compounds found in chicory extract which may be effective on the performance of calcium Glutamate-ergic system (Garavand *et al.,* 2010). Glycosides are organic, light weight compounds consisting of an Aglycen attached to a sugar. Glycosides vary from Glucose due to the replacement of one of the organic bonds with one of glucose's original atoms (Garavand *et al.,* 2010). Glycosides have been known to inhibit the activity of hippocampus through inhibition of Glutamate-ergic cells. In addition, analysis of cortical electroencephalograms of mice, performed by Lu *et al.,* (2005), show that the inhibitory effects of jujuboside A and diazepam mainly take place by inhibiting the effects of Glutamate (Lu *et al.,* 2005). In addition, glycosides increase the expression rate of GABA receptor subunits in the hippocampus reducing neural activity. In this accordance and as the result of a study performed by Ma *et al.,* (2008), it was determined that another glycoside named sanjunin, present in the seeds of Jujube, maybe used as an anti-epileptic drug since it reduces calcium flow and inhibits neural activity. It is highly probable that this effect is mediated by neurotropic Glutamate receptors. Reduction of intracellular calcium concentration in some animal models has shown to reduce and even inhibit epilepsy in these subjects (Ma *et al.,* 2008). As it was mentioned previously, reduction of intracellular calcium inhibits epileptic seizures (Alcaraz and Hoult, 1985). Since flavonoids may be found in excessive amounts in Chicory, it is assumed that the antiepileptic effects of this extract take place through affecting calcium canals (Olivera, 2008; Garavand *et al.,* 2010).

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