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The effect of hydro-alcoholic extract of Cichorium intybus leaf on PTZ-induced seizure in male mice

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ABSTRACT

Background: Regarding to chronic nature of epilepsy, side effects and resistance to chemical drugs, and with the objective to access effective treatment procedures, herbal medicine have received remarkable interest. The aim of this study was to determine the anticonvulsant effects of hydro-alcoholic extract of Cichorium intybus leaves on PTZ-induced seizure in male mice.

Methods: In this study, 56 albino male mice weighing 20-25 g were divided randomly into seven groups. All groups were injected intraperitoneally. The negative and positive control groups received saline (10 ml/1000g) and Phenobarbital (40mg/kg) respectively. Treatment groups received hydro – alcoholic extract of Cichorium intybus leaves at doses of 100, 300, 500, 800 and 1000 mg/kg. All injections were carried out 45 minutes prior to the experiment. In order to provoke convulsion, pentylenetetrazol (PTZ) was injected (80 mg/kg) to all groups after 45 minutes and initiation time of myoclonic and tonic-clonic seizures and death percent after 24 h were measured.

Results: The results indicated that hydro-alcoholic extract delayed the initiation time of myoclonic and tonic-clonic seizures in comparison with control group. The delay was significant at doses of 1000 and 800 mg/kg ($P < 0.001$) and 500 mg/kg ($P < 0.01$) for myoclonic seizure and 1000, 800 and 500 mg/kg ($P < 0.001$) and 300 mg/kg ($P < 0.05$) for tonic-clonic seizure. Also, the extract decreased the 24 h death.

Conclusion: It seems the hydro-alcoholic extract of Cichorium intybus have decremental effect on PTZ-induced seizure.

Keywords: Cichorium intybus; Pentylenetetrazol; Seizure; Mice

Introduction

The term seizure refers to a transient alteration of behavior due to the disordered, synchronous, and rhythmic firing of brain neurons' populations. Epilepsy is the occurrence of unpredictable periodic and recurrent seizures [1], which involves about 0.5 up to 1% of the world's population [2]. People with epilepsy suffer from

sensory-motor, cognitive, psychological and social disorders whose quality of life is impaired and their risk of premature death is threatening [3]. Although epilepsy affects patients at different ages, however it has a more severe effect on the lives of children, the elderly, and people with lower socioeconomic status. In the United States, about $ 15.5 billion is spent directly and indirectly on epilepsy annually [4].

Standard treatments control seizures in a large number of patients, but a high percentage of patients develop uncontrolled epilepsy despite receiving medical treatments [2]. Regarding to chronic nature of epilepsy, side effects, its resistance to chemical drugs, and with the objective to access more effective treatment procedures, herbal medicine have received remarkable interest.

Cichorium intybus(CCI) L. or Chicory is a medicinally important plant that belongs to the Asteraceae family. In traditional medicine, all parts of the plant specially root and leaves are used as diuretic, laxative, antibilious, antipyretic, blood purification and strengthening of the stomach. Few studies have found some important constituents in chicory such as caffeic acid derivatives, fructo-oligosaccharides, flavonoids, inulin, polyphenol, flavonenes and their glycosides, anthocyanins and their glycosides, sesquiterpenes, steroids, triterpenes, and benzo-isochromenes. It also contains a bitter glycoside named cichorine. The sesquiterpene lactones such as lactucin and lactucopicrin have also been isolated from chicory [6, 7, 8]. Also, according to some reports cichorium intybus has antioxidant and anti-inflammatory effects [9].

Due to the antiepileptic properties of flavonoids, antioxidants and anti-inflammatory materials and cichorium intybus enrichment of the mentioned constituents, the aim of this study was to evaluate the anticonvulsant effects of hydro-alcoholic extract of Cichorium intybus leaves on PTZ-induced seizure in male mice.

Methods

Chemicals and Plant Extraction

Pentylenetetrazol (PTZ) and Phenobarbital were purchased from Sigma-Aldrich (St. Louis, MO, USA) and (Desitin, Germany), respectively. After cleaning and drying the plant leaves in room temperature, leaves powder (100 g) was macerated in ethanol 80% for 24 h at dark. Then, the mixture was filtered and concentrated under reduced pressure at 40 ºC by rotary evaporator to prepare dry powder. Next, the extract powder was weighed and diluted by physiological serum to make the suspension. The injection volume was 10 ml per kg body weight of mice.

Animals

In this experimental study, fifty-six albino male mice weighing 20-25 g were obtained from the animal house of School of Medicine, Guilan University of Medical Sciences, Guilan, Iran. The animals were housed in the standard cages with free access to food and water. The temperature of animal house was 22 ± 2 ºc with a 12 hr light/dark cycle. The animals were randomly divided into seven groups (n=8). All groups were injected intraperitoneally (i.p). Negative control group received normal Saline (10 ml/kg). Positive control group received Phenobarbital (40 mg/kg). Experimental groups received hydro- alcoholic extract of Cichoirum intybus at doses of 100, 300, 500, 800 and 1000 mg/kg, respectively. All groups were injected 45 min before administration of PTZ [10]. All animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (Publication No.: 85-23 revised in 1985). All protocols were also approved by ethical and supervisory guidelines of working with experimental animals of Guilan University of Medical Sciences (Code No. IR.GUMS.REC.1394.565).

Seizure Induction

In order to induce experimental model of epilepsy, PTZ (80 mg/kg) was dissolved in normal saline (0.9%) and injected intraperitoneally 45 min after administration of saline, phenobarbital and different amounts of hydro-alcoholic extract of CCI (100, 300, 500, 800 and 1000 mg/kg). The animals were controlled after PTZ injection for 30 min. Then, the latency of myo-clonic and tonic-clonic seizures and also the percent of mortality within 24 h were evaluated.

Statistical Analysis

Data were expressed as mean ± SEM in this study. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey's test (t-test) for multiple comparisons. Protective effects of hydro-alcoholic extract of CCI against mortality after 24 h were evaluated by the Fisher's Exact test. $P < 0.05$ was considered statistically significant.

Results

A: Effect of hydro-alcoholic extract of CCI on latency to myo-clonic Seizure

All animals in negative and treatment groups revealed seizure after PTZ administration. The results showed that all concentrations of extracts increased the latency to myo–clonic seizures in comparison with control group (normal saline). This increase was significant at doses of 1000 and 800 mg/kg ($P < 0.001$) as well as 500 mg/kg ($P < 0.01$), respectively. Also, the increase of myo-clonic seizure latency was significant at doses of 1000 and 800 mg/kg of extract compared to 300 mg/kg ($P < 0.01$) and 100 mg/kg of extract ($P < 0.001$ and $P < 0.01$), respectively (Fig.1).

B: Effect of hydro-alcoholic extract of CCI on latency to tonic-clonic seizure

As shown in Fig.2, all extract amounts increased the latency to tonic–clonic seizures in comparison with saline control group. The increase of latency was significant at doses of 1000, 800 and 500 mg/kg ($P < 0.001$) and 300 mg/kg ($P < 0.05$), respectively. Also, the doses of 800 and 1000 mg/kg of extract significantly increased the latency to tonic-clonic seizure compared to 100 mg/kg of extract ($P < 0.001$ and $P < 0.01$) , respectively.

C: Protective effect of hydro-alcoholic extract of CCI against mortality after PTZ -induced seizure

Hydro-alcoholic extract of CCI decreased the mortality percentage within 24 h compared to control group. The protective effect against mortality (convulsion survivors/ animals tested) of hydro-alcoholic fraction was 37.5% at a dose of 1000 mg/kg and 12.5% at the doses of 100 - 800 mg/kg. The mortality rate comparison among experimental groups was not significant. Phenobarbital completely inhibited PTZ-induced seizures; therefore, the percent of survival of animals after 24 h was %100 (table 1).

Discussion

In the present study, hydro-alcoholic extract of CCI was effective on reduction of PTZ -induced seizure. Hence, the results of this study could underlie its traditional use in treatment of convulsive disorder [11]. We used hydro-alcoholic extract of Cichurium intybus to obtain more amounts of active components compared to water extract in accordance with the results of Jana and colleagues [12]. , extract administrated 45 min before induction of chemical convulsions prior peritoneal absorption [10].

According to our findings, CCI hydro-alcoholic extract antagonized PTZ effect in a dose response manner based on the increase of latency of myo-clonic and tonic-clonic seizures in comparison with control group.

There are some experiences regarding the anticonvulsant effects of CCI extract against PTZ-induced seizure [13, 14, 15] in line with our study. The novelty of our study was the usage of different doses of chicory, the time difference between chicory injection and pentylenetetrazol injection to induce seizures and measurement of different seizure indices.

Terpenes have been isolated from Cichorium intybus with the aid of phytochemical screening [7, 8]. Monoterpenes such as carvone and limonene have protective effect against PTZ-induced convulsion [16, 17]. Also, Flavonoids and their derivatives have been found in Cichorium intybus [7,8]. Flavonoids are an important class of natural compounds exert anti-oxidant properties [18]. Since, chemical seizure induced by PTZ responds to antioxidant compound; flavonoids in extract may potentially be involved in anti-convulsant outcome [19].

Flavonoids have several neuropharmacological activities. Some of these effects are related to γ-aminobutyric acid type A ($GABA_A$) receptors in the central nervous system [20]. In addition, flavonoids are able to potentiate the effect of GABA on its receptors by crossing the blood-brain barrier and acting as a positive allosteric regulator [21]. It has also been reported that flavonoids reduce the calcium entry into the cell

and reduce the concentration of intracellular calcium by acting on the NMDA receptor and inhibiting their activity [22].
Therefore, it seems that the flavonoid compounds in chicory leaves are effective in anticonvulsant effects.

As, PTZ induces convulsion by antagonizing ($GABA_A$) receptor chloride channel complex, manipulation of ($GABA_A$) receptors by flavonoids, in turn, affect on CNS activity [23].

glycosides are one of the chicory compunds [24] which may reduce calcium intake by acting on the glutamatergic system [21].There are some evidences that manifest the inhibitory effect on calcium L- type channels which are involved in convulsion [25, 26]. This antagonistic effect in this study may partially be explained by decreasing the seizure obtained initiation time.

Cyclooxygenase (COX) is reported to play a significant role in neurodegenerative and neuropsychiatric disorders, and may have a prominent place in the pathogenesis of epilepsy. some reports regarding the possible role of cyclooxygenase isoenzymes in the pathophysiology of epilepsy and the use of COX-inhibitors as an adjuvant therapy in the treatment of epilepsy exist [7]. Cyclooxygenase 2 activity increases oxidative stress by increasing the production of free radicals and induces apoptosis of GABAergic neurons. Therefore, by removing the inhibitory effect of GABA on glutamatergic neurons, the tone of glutamatergic neurons increases and therefore seizures enhances. Epileptic seizures have been reported to be controlled by inhibiting cyclooxygenase 2 and reducing glutamate secretion [21, 27].
Chicory inhibits this chain by inhibiting cyclooxygenase 2 and suppresses seizures by strengthening the GABAergic system and inhibiting the glutamate system.

Ethyl acetate chicory extract inhibits the producrion of prostaglandin E(2) (PGE(2)) in human colon carcinoma HT29 cells treated with the pro-inflammatory agent TNF-alpha. In an experiment two independent mechanisms of action wereidentified: (1) a drastic inhibition of the induction by TNF-alpha of cyclooxygenase 2 (COX-2) protein expression and (2) a direct inhibition of COX enzyme activities with a significantly higher selectivity for COX-2 activity. The inhibition of TNF-alpha-dependent induction of COX-2 expression was mediated by an inhibition of NF-kappa B activation. Sesquiterpene lactone of chicory and guaianolide 8-deoxylactucin were identified as the key inhibitor of COX-2 protein expression present in chicory extract [28].

In line with some previous reports, our findings suggest that the anti-inflammatory effect of CCI might be linked with reduced expression of proinflammatory cytokines, such as TNF-α and NF-κB, in ET2D rats [9]. TNF-α is produced in the early stages of inflammation and controls the production of other cytokines. It is expressed as a 26-kDa cell surface transmembrane protein and after its activation cleaves to a 17-kDa soluble form [29]. According to some studies sesquiterpene lactones inhibit pro-inflammatory gene expression through inactivation of the transcription factor nuclear factor-kappa B (NF-kB) [30, 31]. Several pro-inflammatory genes including those coding cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), inducible nitric oxide synthase (iNOS) and interleukin 1, beta (Il1β) contain a binding site in their promoter region for NF-kB [32], therefore, their expression could be mediated through the NF-kB pathway. Several sesquiterpene lactones were isolated in an experiment by () from chicory extract that could be responsible for anti-inflammatory activity. In this experiment data suggested that chicory extract was an effective anti-inflammatory agent that could be developed as a functional food, nutraceutical, or pharmaceutical intended to prevent and treat various inflammatory conditions [33].
This antioxidant property of chicory is related to its polyphenolic compounds [34]. The duration of tonic-clonic seizures report was the encountered limitation of our study. This was due to high mortality in animals receiving the PTZ.

Conclusion

H-alcoholic extract of Cichurium intybus demonstrated anticonvulsant activity against PTZ-induced seizure. It might be considered as an adjuvant therapy with other traditional antiepileptic medications. Nevertheless, further studies are necessary to elucidate the involvement of probable neurotransmitter which mediates the functional mechanisms of whole extract.

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Author Contributions: HGh contributed to the execution of test, data collection & writing; MR contributed to the design, analysis, and interpretation of data and writing & editing the draft

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