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Original Research Article

Potential Anticonvulsant Activity of Ethanol Extracts of *Cichorium intybus* and *Taraxacum serotinum* in Rats

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Abstract

Purpose: *To evaluate the acticonvulsant activity of Cichorium intybus (C. intybus) and Taraxacum serotinum (T. serotinum) in maximal electroshock (MES), as well as pentylenetetrazole (PTZ)- and strychnine nitrate (STN) - induced seizure models in rats.*
Methods: *For each model, 8 groups of Swiss albino rats (n=10) were used. The 1st group was kept as control, 2nd as standard (diazepam, 7.5 mg/kg); 3rd - 5th were treated with C. intybus ethanol extract (125, 250 and 500 mg/kg); and 6th - 8th treated with T. serotinum extract (125, 250 and 500 mg/kg). After 30 min of administration, the rats were exposed to a shock of 150 mA by a convulsiometer, via ear electrodes for 2 s (in MES test) or sc injection of PTZ (85 mg/kg) or STN (2.5 mg/kg). Anticonvulsant activity was confirmed by abolition of hind limb tonic extension (HLTE) in MES test and by measuring the latency to PTZ or STN-induced threshold seizures, and the duration of seizures in the rats.*
Results: *In MES model, 500 mg/kg of C. intybus and T. serotinum resulted in complete abolition of HLTE in 70 and 50 % of the rats, respectively, compared to 80 % in diazepam-medicated animals. Both extracts at 500 mg/kg prolonged latency to seizure onset in PTZ model to 144.7 and 114.7 s, respectively (vs 55.2 s in control group; $p < 0.05$). Both extracts failed to protect rats against STN-induced seizures.*
Conclusion: *C. intybus and T. serotinum possess anticonvulsant effect as they both abolish HLTE induced by MES and delay the latency of seizures produced by PTZ.*

Keywords: *Cichorium intybus, Taraxacum serotinum, Anticonvulsant, Seizures, Maximal electroshock, Pentylenetetrazole, Strychnine nitrate*

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INTRODUCTION

Despite fundamental progress made in the treatment of neurological disorders, epilepsy remains a significant therapeutic defiance. Epilepsy, a disorder of the brain is a major health problem that affects 1–2 % of the world population [1]. It is characterized by recurrent spontaneous seizures, in addition to unpredictable and periodic occurrence of a transient alteration of behavior due to the disordered, synchronous and rhythmic firing of populations of brain neurons. It has been observed that presently available antiepileptic drugs do not provide cure nor prevent relapse and they are often associated with debilitating adverse effects [2]. Toxicity, intolerance, and lack of efficacy are the limitations of the current antiepileptic drugs. The development of new pharmacological agents that can overcome these barriers has become a major goal in epilepsy research. The plant kingdom is a major target in the search of new drugs of natural origin to be used for protection against this debilitating neurological disorder.

Cichorium intybus L. or Chicory is a medicinally important plant that belongs to the family Asteraceae. The genus *Cichorium* consists of six species with major distribution areas in Europe and Asia. Asteraceae is the largest family of flowering plants, comprising about 1,100 genera and more than 20,000 species. Chicory lives as a wild plant on roadsides in its native Europe, and in North America and Australia, where it has become naturalized. It is known in Turkey as catlangaç, çatlangaç süpürgesi, Taşlik Badik out and Çıtlak out. Its habitats are roadsides, railroads and waste grounds. 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 [3]. It is also used as an appetizer as well as in the treatment of hepatic failure, jaundice, intermittent fever and mild states of chronic skin diseases. Recent studies have found some of the important constituents in chicory such as caffeic acid derivatives, fructooligosaccharides, flavonoids, inulin, and polyphenol [4]. It also contains a bitter glycoside named cichorine. The sesquiterpene lactones such as lactucin and lactucopicrin were isolated from chicory and reported for its antibacterial and antimalarial activity [5].

The genus *Taraxacum* is a member of the family Asteraceae and widely distributed in the warmer temperate zones of the Northern Hemisphere. The perennial weed has been known since ancient times for its curative properties and has been utilized for the treatment of various ailments such as dyspepsia, heartburn, spleen and liver complaints, hepatitis and anorexia [6]. Several flavonoids including caffeic acid, chlorogenic acid, luteolin, and luteolin 7-glucoside have been isolated from plants of the genus *Taraxacum* [7]. *T. serotinum* plant is known in Turkey as sütlük and is considered as one of the vitamin-rich plants. The leaves of *T. serotinum* are used in the Turkish folk medicine as cardiotonic [8]. The plant is also used as an appetizer and digestant.

Some plants of Asteraceae are used widely in Africa and Asia for the treatment of epilepsy. This study was undertaken to evaluate the possible anticonvulsant activities of *C. intybus* and *T. serotinum* extracts using different *in vivo* models such as maximal electroshock seizure (MES), as well as pentylenetetrazole (PTZ) and strychnine (STN)-induced seizure tests.

EXPERIMENTAL

Plant material

Fresh flowering stage aerial parts of *Cichorium intybus* L. and *Taraxacum serotinum* (Wldst. & Kit.) were collected during summer, 2010 from Beypazarı-Nallıhan way, Ankara , Turkey and Hasanoğlan, surrounding Yeşildere village, Ankara, Turkey, respectively. Both plants were identified by Prof. Dr. Galip Akaydin and voucher specimens (Akaydın 10301 and Akaydın 13430, respectively) were deposited at Hacettepe University, the Herbarium of Faculty of Education (HEF), Ankara, Turkey.

Experimental animals

Twenty one-day-old Albino rats of either sex were obtained from the Animal House of the National Research Centre, Cairo, Egypt. Animals were maintained under standard conditions of temperature (23 ± 1.0 °C), humidity (55 ± 10 %), and 12 h light/12 h dark cycle and fed with a standard pellet diet (National Company for Animals' diet, Dakahleya, Egypt) with water *ad libitum*. Rats were kept in groups of ten in standard polypropylene cages. Animals were allowed to acclimatize to the laboratory environment for one week before experimentation. No rat was used more than once for the investigation of the anticonvulsant effect of the tested extracts. Animal procedures were performed in accordance with the Ethics Committee of the National Research Centre and followed the recommendations of the Institutes for Laboratory Animal Research [9].

Preparation of plant extract

The collected plants were shade dried and then grinded to fine powder. The dried powder of each plant (400 g) was extracted by percolation in 70 % aqueous ethanol with occasional shaking for 48 h. Percolation was repeated three times, and then the ethanolic extracts of each plant were combined, filtered and concentrated to dryness under reduced pressure at 60 ± 1 °C in a rotary evaporator to give the total extracts of *C. İntybus* and *T. serotinum*. Both extracts were stored in the refrigerator and aliquot of the concentrations were prepared immediately before use.

Acute toxicity (LD_{50}) test

The oral median lethal doses (LD_{50}) of the extracts were determined in Albino rats as described by Lorke [10]. Briefly, each extract was administered orally at different ascending doses (50-5000 mg/kg) to groups of three rats each. Control animals received the solvent (3 % v/v Tween 80 in distilled water) and were kept under the same conditions without any treatment. Animals were observed for 24 h for signs of toxicity and mortality. The LD_{50} value was determined by calculating the geometric means of the lowest dose that caused death and the highest dose for which the animals survived.

Justification for dose selection

C. İntybus and *T. serotinum* extracts were nontoxic at the dose of 5000 mg/kg. Accordingly, experimental doses of 125, 250 and 500 mg/kg that are equal to 1/40, 1/20 and 1/10 of the highest possible dose tolerated by rats were selected for the study.

Screening for anticonvulsant activity

Both the electrically induced seizure model (MES) and chemically induced seizure models (scPTZ and scSTN) were used to determine the anticonvulsant activity of *C. İntybus* and *T. serotinum* extracts in 21-day-old Albino rats.

Maximal electroshock seizure (MES) test

The MES test was performed on 21-day-old Albino rats of either sex by following the method of Garg *et al* [11]. Animals were divided into 8 groups each consisting of ten rats and treated for 10 days. Group I received the vehicle (3 % Tween 80, orally); group II was allotted for standard drug (diazepam, 7.5 mg/kg, ip). Groups III, IV and V received *C. intybus* extract at oral doses of 125, 250 and 500 mg/kg, respectively. Groups VI, VII and VIII received *T. serotinum* extract at oral doses of 125, 250 and 500 mg/kg, respectively. On the 10th day, 30 min after administration of the last dose of the vehicle, extracts and diazepam, rats were subjected to a shock of 150 mA by convulsiometer (ECT unit, model 57800, Ugo Basile, Comerio, Italy), through ear electrodes for 2 sec. The number of animals exhibiting hind limb tonic extension (HLTE) seizures and the percentage of animals protected against HLTE were recorded. Animals in which HLTE response were abolished within 10 sec after delivery of the electroshock were taken as protected rats. The HLTE was judged abolished if the extension of hind limb did not exceed a 90° angle with the plane of the body.

Pentylenetetrazole (PTZ) and strychnine (STN)-induced seizure tests

For each chemoconvulsant, 80 rats were used (n=10). Grouping and dosing patterns were similar to those stated with MES test. Rats were administered the vehicle, diazepam and the test extracts for ten days. On the 10th day, 30 min after administration of the last dose of the vehicle, diazepam and the test extracts, seizures were induced in rats by subcutaneous injection of PTZ (85 mg/kg, for PTZ-induced seizure test)or STN (2.5 mg/kg, for STN-induced seizure test). The latency to PTZ or STN-induced threshold seizures, the duration of seizures, percentage of animals protected against seizures and percentage of animals protected against lethality were recorded within a thirty minute period [12].

Statistical analysis

Data were expressed as percentage (%) protection and mean ± SEM and were analyzed by one-way ANOVA followed by Dunnett's test for multiple comparisons using the SPSS version 10. Results were considered significant at $p < 0.05$.

RESULTS

Acute oral toxicity

The results obtained indicated that *C. intybus* and *T. serotinum* extracts at oral doses up to 5000 mg/kg did not produce any symptom of acute toxicity and none of the rats died during 24 h of observation. All animals did not exhibit diarrhea, haematuria, restlessness, uncoordinated muscle movements, and respiratory distress accordingly; it suggested that oral median lethal dose (LD_{50}) of the tested extracts were higher than 5000 mg/kg b.wt.

Anticonvulsant activity

Treatment of rats with the ethanolic extracts of *C. intybus* (125 mg/kg) and *T. serotinum* (125 and 250 mg/kg), did not produce significant effect against seizure induced by MES. They failed also to protect the rats against seizure induced by PTZ. In addition, both extracts at all tested doses failed to protect rats against STN-induced seizure.

In the maximal electroshock seizure (MES) test, 100 % of the controlled rats exhibited hind limb tonic extensions (HLTE) seizure. Diazepam at the dose of 7.5 mg/kg provided 80 % protection against MES seizure. In addition, the ethanolic extracts of *C. intybus* and *T. serotinum* demonstrated dose-dependent anticonvulsant activity against electroshock-induced HLTE. *C. intybus* extract at the doses of 250 and 500 mg/kg provided 60 % and 70 % protection, respectively while *T. serotinum* (500 mg/kg) provided 50 % protection (Table 1).

PTZ-induced clonic convulsions as well as lethality in rats, whereas pretreatment of the rats with *C. intybus* (250 and 500 mg/ kg) and *T. serotinum* (500 mg/ kg) extracts caused significant delay in the onset of the seizure as well as lethality (Table 2). Both doses of *C. intybus* delayed the latency to PTZ-induced threshold seizure to 116.4 ± 6.56 and 144.7 ± 7.82 s, respectively, compared with normal controls (55.2 ± 2.58 s).

In the PTZ induced seizure model, diazepam (7.5 mg/kg) inhibited the tonic seizures and death both at 100 %. In comparison, *C. intybus* extract (250 and 500 mg/kg) showed protection against seizure (50 and 60 %, respectively) and mortality (60 and 90 %, respectively). While *T. serotinum* (500 mg/kg) showed protection against PTZ induced seizure and prevented death both at the rate of 50 %.

In this study, it was observed that *C. intybus* and *T. serotinum* extracts did not produce significant anticonvulsant effect against STN-induced seizures as compared to the control (Table 3).

DISCUSSION

In screening natural products for pharmaco-logical activity, assessment and evaluation of the toxic characteristics of a natural product extract, fraction, or compound are usually initial steps taken. In this study, *C. intybus* and *T. serotinum* extracts at doses up to 5000 mg/kg had no treatment-related signs of toxicity or mortality in any of the animals tested during 24 hours of observation. The LD_{50} of *C. intybus* and *T. serotinum* was therefore estimated to be more than 5000 mg/kg. Therefore, *C. intybus* and *T. serotinum* can be categorized as highly safe extracts since substances possessing LD_{50} higher than 50 mg/kg b.wt are non-toxic [13].

Table 1: Anticonvulsant activity of *C. intybus* and *T. serotinum* extracts in MES-induced seizure in rats

Treatment	Dose (mg/kg)	No. of animals exhibiting seizures	Protection against seizures (%)
Control (vehicle)	5 mL/kg	10/10	0
Diazepam	7.5	2/10	80
	125	9/10	10
C. intybus	250	4/10	60
	500	3/10	70
	125	9/10	10
T. serotinum	250	8/10	20
	500	5/10	50

The results are expressed ratio and percentage (n =10)

Table 2: Anticonvulsant activity of *C. intybus* and *T. serotinum* extracts in PTZ-induced seizure in rats

Treatment	Dose (mg/kg)	No. of animals exhibiting seizures	Latency (s)	Duration of seizures (s)	Protection against seizures (%)	Protection against lethality (%)
Control (vehicle)	5 mL/kg	10/10	55.2±2.58	192.5±8.72	0	0
Diazepam	7.5	0/10	ND	ND	100	100
	125	9/10	61.8±3.60	184.2±7.27	10	0
C. intybus	250	5/10	116.4±6.56*	113.5±4.57*	50	60
	500	4/10	144.7±7.82*	93.2±3.95*	60	90
	125	9/10	58.2±2.73	185.7±8.16	10	0
T. serotinum	250	8/10	74.6±6.35	172.4±5.64	20	10
	500	5/10	114.7±5.85*	114.4±4.24*	50	50

*The results are expressed as mean ± S.E.M. and %, n = 10 rats/group; * indicate significance compared to normal control group at p< 0.05 (Dunnett's test); ND = not determined*

Table 3: Anticonvulsant activity of *C. intybus* and *T. serotinum* extracts in STN-induced seizure in rats

Treatment	Dose (mg/kg)	No. of animals exhibiting seizures	Latency (s)	Duration of seizures (s)	Protection against seizures (%)	Protection against lethality (%)
Control (vehicle)	5 mL/kg	10/10	115.4±6.15	37.6±1.25	0	0
Diazepam	7.5	0/10	ND	ND	100	100
	125	10/10	121.3±6.73	35.6±1.75	0	0
C. intybus	250	10/10	134.6±6.83	33.2±1.26	0	0
	500	8/10	142.2±6.12	32.3±1.27	20	0
	125	10/10	120.3±5.37	36.9±1.32	0	0
T. serotinum	250	10/10	127.6±5.75	33.8±1.63	0	0
	500	9/10	136.2±5.33	33.3±1.66	10	0

The results are expressed as mean ± SEM and %, n =10 rats/group; ND = not determined

According to the chemical labelling and classification of acute systemic toxicity recommended by the Organization for Economic Cooperation and Development (OECD), the ethanolic extracts of *C. intybus* and *T. serotinum* were assigned class 5 status (LD_{50} > 5000 mg/kg), which is the lowest toxicity class. In addition, substances with LD_{50} values higher than 5000 mg/kg by oral route are regarded as being safe or practically nontoxic [14].

Despite the diversity of models that could potentially be used to screen for anticonvulsant activity, the maximal electroshock seizure (MES) and the subcutaneous pentylenetetrazole (PTZ)-induced seizure models remain ‘Gold standards’ in the early stages of testing. In the present investigation, the tested extracts were subjected to a screening in anticonvulsant assays including MES, PTZ and strychnine (STN)-induced seizure tests in 21-day-old Albino rats. Development of a new compound for the treatment of epilepsy relies heavily on the use of predictable animal models.

The present results indicate that the ethanolic extracts of *C. intybus* (250 and 500 mg/kg) and *T. serotinum* (500 mg/kg) have anticonvulsant activities in the MES and PTZ-induced seizure tests. It has often been stated that anticonvulsant drugs that prevent tonic extension of MES act by blocking spread of seizure whereas drugs that either prevents or delays seizure of PTZ, act by elevating the seizure threshold [15].

In the maximal electroshock seizure (MES) test, 100 % of the controlled rats exhibited hind limb tonic extensions (HLTE) seizure. The MES is a standard procedure that evaluates the ability of the testing materials to protect against HLTE. Ibrahim *et al* [16] stated that the seizure features in MES are similar for all laboratory animals and human except for the time scale. The standard drug diazepam (7.5 mg/kg) and the ethanolic extracts of *C. intybus* (250 and 500 mg/ kg) and *T. serotinum* (500 mg/ kg) exhibited significant anticonvulsant activity and offered 80, 60, 70, and 50 % protection against electroshock-induced HLTE, respectively. In the MES, protection against HLTE predicts the anticonvulsant activity of the tested agents. Moreover, protection against HLTE in MES-induced seizure indicates the capability of *C. intybus* and *T. serotinum* extracts to either stop or to slow down the discharge of the seizure within the brain stem substrate [17].

Seizure of MES can be blocked either by inhibiting the voltage-dependent Na^+ channels or by blocking glutamatergic excitation mediated by the N-methyl-D-aspartate (NMDA) receptors [18]. Since *C. intybus* and *T. serotinum* extracts showed anti-epileptic activity in the MES, they may act by inhibiting the voltage-dependent Na^+ channels or by blocking the glutamatergic neurotransmission mediated by NMDA receptors. In addition, various classes of phytoconstituents such as flavonoids, phenols, and terpenes have been reported to possess anticonvulsant activity [19]. Accordingly, the significant anticonvulsant activities of *C. intybus* and *T. serotinum* extracts may be due to the presence of many potent compounds such as flavonoids [7].

In PTZ test, diazepam (7.5 mg/kg), *C. intybus* (250 and 500 mg/kg) and *T. serotinum* (500 mg/kg) extracts exhibited a significant anticonvulsant effect. *C. intybus* was found to be more effective than *T. serotinum*. These results provide evidence that both extracts possesses anticonvulsant activity. The ability of both extracts to delay the onset of convulsions and/or shorten the duration of convulsions was considered an indication of anticonvulsant activity.

It was suggested that compounds which are effective in suppression of PTZ-induced clonic seizures partially overlapped with the group of compounds effective against MES [20]. In this regard, diazepam was found to be more effective against PTZ than MES seizures which agrees with the idea that PTZ is a GABA-A receptor antagonist. Accordingly, PTZ produces seizures by blocking the major GABAergic inhibitory pathways in the central nervous system [21]. Standard antiepileptic drugs such as diazepam are thought to produce their effects by enhancing GABA-mediated inhibition in the brain [22]. Moreover, activation of the N-methyl-d-aspartate (NMDA) receptors is also involved in the initiation and propagation of PTZ-induced seizures [23]. In this regard, drugs that block glutamatergic excitation mediated by NMDA receptors have demonstrated anticonvulsant activity against PTZ-induced seizures [22]. Seizures induced by PTZ can also be blocked by reducing T-type Ca^{2+} currents [24]. Therefore, the anticonvulsant activities of *C. intybus* and *T. serotinum* extracts against PTZ seizures might be due to an enhancement on the release of the inhibitory neurotransmitter GABA in the central nervous system, inhibiting T-type Ca^{2+} currents or blocking the glutamatergic neurotransmission mediated by NMDA receptors, which were not tested in this study.

The convulsive action of strychnine is due to interference with postsynaptic inhibition mediated by glycine, an important inhibitory transmitter to motor neurons and interneurons in the spinal cord [25]. In this study, it was observed that both extracts did not produce significant anticonvulsant effect against STN-induced seizures as compared to control, suggesting their inability to interact with the glycine-mediate inhibitory pathway.

CONCLUSION

C. intybus and *T. serotinum* extracts show marked protective activities against PTZ-induced and maximal electroshock seizures, but are ineffective against STN-induced convulsion. Further phytochemical studies are required to isolate and identify the active molecule(s) responsible for anticonvulsant activity.

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