

# The Electrophysiological Consequences of *Artemisia dracunculus* L. (Tarragon) Extract on Pentylenetetrazol-Induced Epileptiform Activity in Snail Neurons

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## Abstract

**Objective:** Plant extracts are of considerable interest because of their antiepileptic activities. However, the mechanisms of action are not clearly defined.

**Materials and Methods:** Here, the effects of *Artemisia dracunculus* L. (tarragon) leaves extract on excitability and electrophysiological characteristics of snail neurones were investigated, using an intracellular recording technique.

**Results:** Application of tarragon extract (0.05%) resulted in complete disappearance of paroxysmal depolarization shift (PDS) as elicited by pentylenetetrazol (PTZ), an epileptogenic drug. It also significantly decreased the firing frequency and shifted the firing pattern from bursting in the presence of PTZ to an irregular doublet activity. Changes in excitability properties were associated with a significant increase and decrease in the duration of action potential, and in the amplitude of after-hyperpolarization (AHP), respectively. When tarragon extract was applied alone, spontaneous activity became irregular and was interrupted by large inhibitory postsynaptic potentials (IPSPs), which disappeared following application of picrotoxin (100  $\mu$ M). Tarragon also caused a significant decrease both in the amplitude of action potentials and AHP, and broadened the action potentials. However, pretreatment with extract did not prevent the induction of epileptiform activity by PTZ.

**Conclusion:** The findings suggest that tarragon extract may affect membrane ion channels and/or GABAA receptors leading to a reduction in neuronal excitability.

**Keywords:** Epilepsy, Action Potential, *Artemisia*, Pentylenetetrazol

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## Introduction

Herbs and spices have been used for generations by humans both as food and to treat ailments. Scientific evidence is accumulating that many of these herbs and spices do have medicinal properties with which to alleviate symptoms or prevent disease (1-3). However, their precise mechanisms of action at the cellular level are mainly unknown. *Artemisia dracunculus* L. (tarragon) is a perennial herb in the family Asteraceae related to wormwood that stimulates the digestive system and uterus, lowers fevers and destroys intestinal worms. The leaves contain about 0.3% essential oil, of which approximately 70% is methyl chavicol (4). The essential oils are used as a food flavoring, in detergents and also medicinally. Ex-

tracts and essential oils from different parts of the tarragon plant have shown radical-scavenging activities (5), in addition to antifungal and antitumor effects (6, 7). In folk medicine, the fruit and dried above-ground parts of this plant have been mentioned as treatments for epilepsy, toothache and diarrhea (8). The plant is mildly sedative (9) and has been taken as an aid to sleep (10). Recently, anticonvulsive activity of the essential oil of tarragon in a rat model of epilepsy has been shown. It is said that the monoterpenoids, particularly trans-anethole, pinene and methyl eugenol which are present in the essential oil mediate its anticonvulsant activity (9).

Epilepsy is one of the most common neurological disorders. Although no definite radical therapy

against epilepsy exists, however actual therapy is based upon the simple inhibition of epileptic activity. There are a number of reports about the use of plants as traditional treatments for epilepsy. However, the mechanisms of their effects at the cellular level are yet unknown.

In addition, invertebrate neuronal cells have often been used as experimental models for studying the action of epileptogenic drugs in order to gain a better understanding of the neurophysiological basis of epilepsy (11, 12). Invertebrate preparations have also been used to determine the mode of action of anticonvulsants on bioelectrical activity (11).

Hence, the purpose of the present study were: 1. to see if the ethalonic extract of tarragon has any antiepileptic effect on experimentally-induced epileptiform electrical discharges; 2. to point out any possible cellular mechanisms of the effects of tarragon extract alone; and 3. to determine the preventative effect of extract, using an intracellular recording technique under current clamp conditions.

## Materials and Methods

### *Animals and dissection*

This study was performed on the soma membrane of neurons from sub-oesophageal ganglia of *Helix aspersa* (Iranian garden snail). The animals were anaesthetized by injection with 2 ml of 50 mM MgCl<sub>2</sub>. The ganglionic mass with its main peripheral nerves and aorta was dissected out and then pinned by the nerve and edges of the connective tissue into a Sylgard 184 grounded recording chamber (Dow Corning Midland, MI, USA). The superficial layers of the connective tissue overlying the ganglia were gently torn using two pairs of forceps without any pre-treatment with proteolytic enzymes. D5 neurons were visually identified by their size and color within the left parietal ganglion (12). The normal snail Ringer's solution contained: 80 mM NaCl, 4 mM KCl, 10 mM CaCl<sub>2</sub>, 5 mM MgCl<sub>2</sub>, 10 mM Glucose and 5 mM HEPES (13). These procedures were undertaken in accordance with the Guidelines of the Institutional Animal Ethics Committee at Shahid Beheshti University of Medical Sciences.

### *Intracellular recording*

A conventional current clamp method was applied using an Axoclamp 2B amplifier (Axon Instrument, Foster City, CA, USA). The reference electrode in all experiments was a silver-silver chloride wire within an agar bridge (4% agar in snail Ringer's solution). The recording chamber, together with micromanipulators and preampli-

fier, were kept in a Faraday cage. The electrophysiological recordings were made in real time by testing spontaneous neuronal activity, before (control), and after application of pentylenetetrazol (PTZ) and tarragon extract. Data were filtered at 30 kHz, voltage records were sampled at 20 kHz and digitized online using a 16 bit A/D converter (AD Instrument Pty Ltd., Sydney, Australia) and stored for further analysis using Chart 5 and Mat-lab softwares. Further analysis was carried out by measuring the action potential parameters, including the amplitude (mV) and duration (mS) of the action potentials, and additionally the amplitude of after-hyperpolarization potential (AHP; mV). The amplitude of the action potential was measured as the sum of the absolute peak value of the action potential and the peak of AHP. The duration of action potential was measured from the point of spike initiation to the point at which repolarization crossed the same voltage; the amplitude of AHP was measured from the resting membrane potential to the end of the spike repolarization.

### *Plant material and drugs*

Fresh above-ground parts of *A. dracunculus* were collected from the plants cultivated in Rey (a city located 20 km south of Tehran), in May 2006. *Artemisia dracunculus* was authenticated by M. Kamalinedjad and a voucher specimen (no. 861) was deposited in the herbarium of the Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences.

### *Extraction*

Plant materials were dried, far from direct sunlight. Then, 100 g of the dried above-ground parts were added to 1 liter of 96% ethanol and kept at room temperature (23-27°C) for 48 hours. The extract was filter evaporated using a rotary evaporator and dried extract was obtained.

### *Materials*

Pentylenetetrazol (PTZ, Sigma) was applied (25 mM) into the bathing solution. An extract of tarragon was dissolved in absolute ethanol at a final concentration of 0.05%. This concentration of the extract was chosen based on preliminary experiments. The final concentration of the vehicle in the perfusion solutions was 0.3% (v/v). The same concentration of vehicle had no effect on bioelectrical activity of the neurons. pH of the solutions was adjusted to 7.8 with either trizma hydrochloride or trizma base (Sigma). Each solution was superfused into the experimental chamber at a rate of approximately 2.5 ml/min.

### Statistical analysis

Numerical results are given as mean  $\pm$  SEM., with  $n$  being the number of cells on which the measurement was performed. Significant differences between the groups were evaluated using student's t-test and one way ANOVA.  $P < 0.05$  was considered to be significant.

### Results

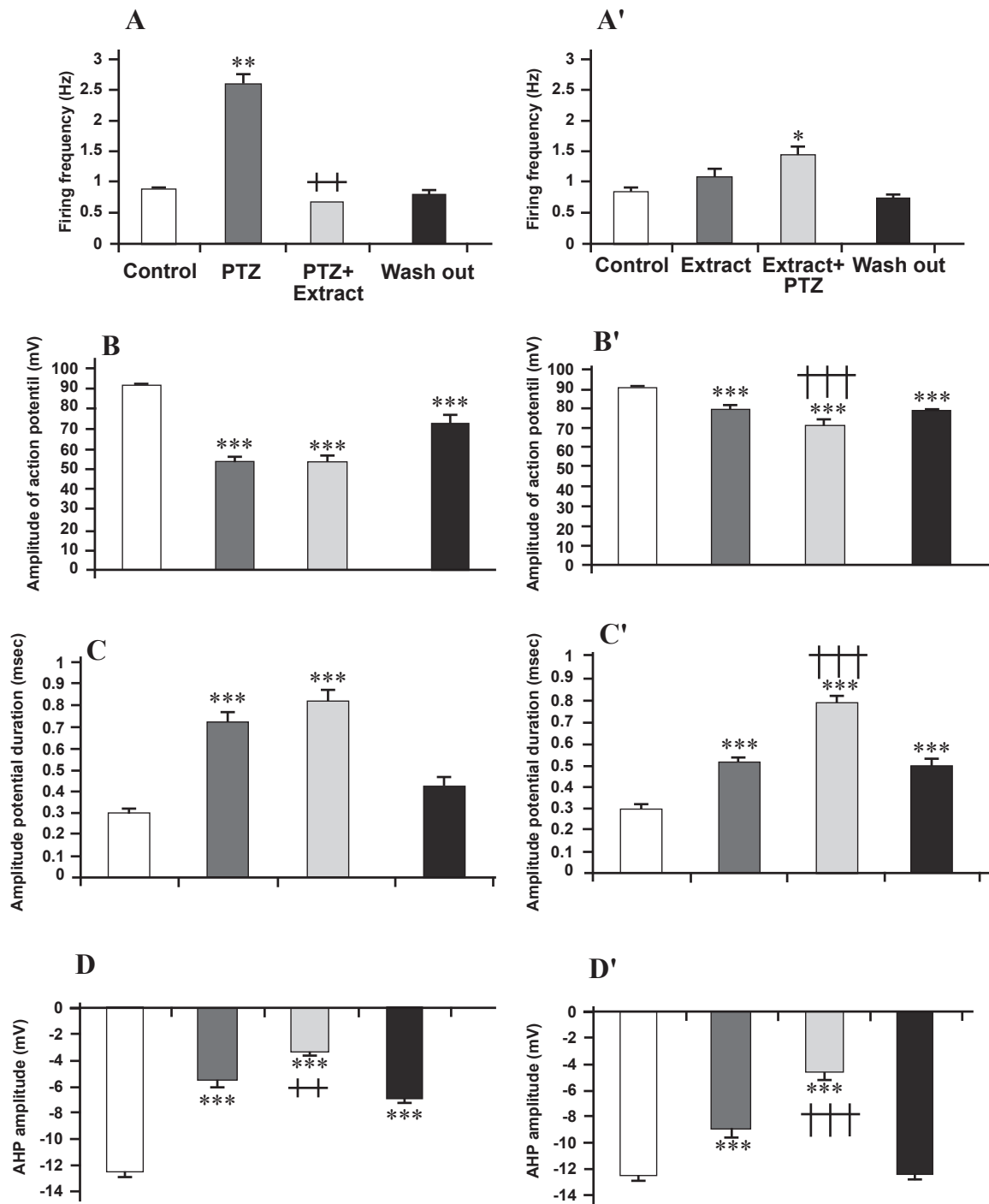
In the first part of this study, the antiepileptic potential of tarragon extract was examined on PTZ induced epileptiform activity. The second part of this study investigated the possible preventative potential of the extract. Conventional intracellular recording, in current clamp mode, was performed to study the functional effects of crude ethanol extract from the above-ground parts of tarragon on PTZ-induced epileptiform activity, action potential configuration and the excitability of D5 neurons ( $n=45$ ) from the posterior region of the left parietal ganglion of *Helix aspersa*. In normal Ringer's solution, the mean resting membrane potential (RMP) and spontaneous action potential frequency (Fig 1A) were  $-45 \pm 2.03$  mV and  $0.87 \pm 0.03$  Hz, respectively. Regularly spaced action potentials (Fig 2A) had a mean amplitude of  $91 \pm 0.6$  mV and a duration of  $0.3 \pm 0.024$  mS ( $n=20$ , Fig 1B and C). Single action potentials were followed by AHP with a mean amplitude of  $-12.59 \pm 0.2$  mV (Fig 1D). When PTZ (25mM) was applied in the perfusion solution, within 5-7 minutes, clear neuronal hyperexcitability with paroxysmal depolarization shift (PDS; noted in Figure 2B by an asterisk) was observed. The firing pattern after 20 minutes of exposure to PTZ changed from a high frequency singlet spiking to a burst pattern (Fig 2C). PTZ treatment also effectively modulated the neuronal firing rate and changed the action potential parameters compared to the control. It slightly depolarized the RMP ( $-43.75 \pm 4$  mV,  $n=20$ ), significantly increased the firing frequency ( $2.61 \pm 0.14$  Hz,  $p < 0.01$ ,  $n=20$ , Fig 1A) and the duration of action potentials ( $0.72 \pm 0.053$ ,  $p < 0.001$ ,  $n=20$ , Fig 1C) as compared with control, but reduced the amplitude of action potentials ( $54.22 \pm 1.81$ ,  $p < 0.001$ ,  $n=20$ ) and AHP that followed action potential ( $-5.5 \pm 0.4$ ,  $p < 0.001$ ,  $n=20$ ; Fig 1B and D). When tarragon extract (0.05 %) was applied under such conditions (in PTZ perfusion), PDS disappeared and the cells fired irregular doublets, interrupted occasionally by quiescent periods associated with inhibitory postsynaptic potentials (IPSPs; Fig 2D).

Furthermore, application of extract in the presence of PTZ caused a significant decrease in the firing

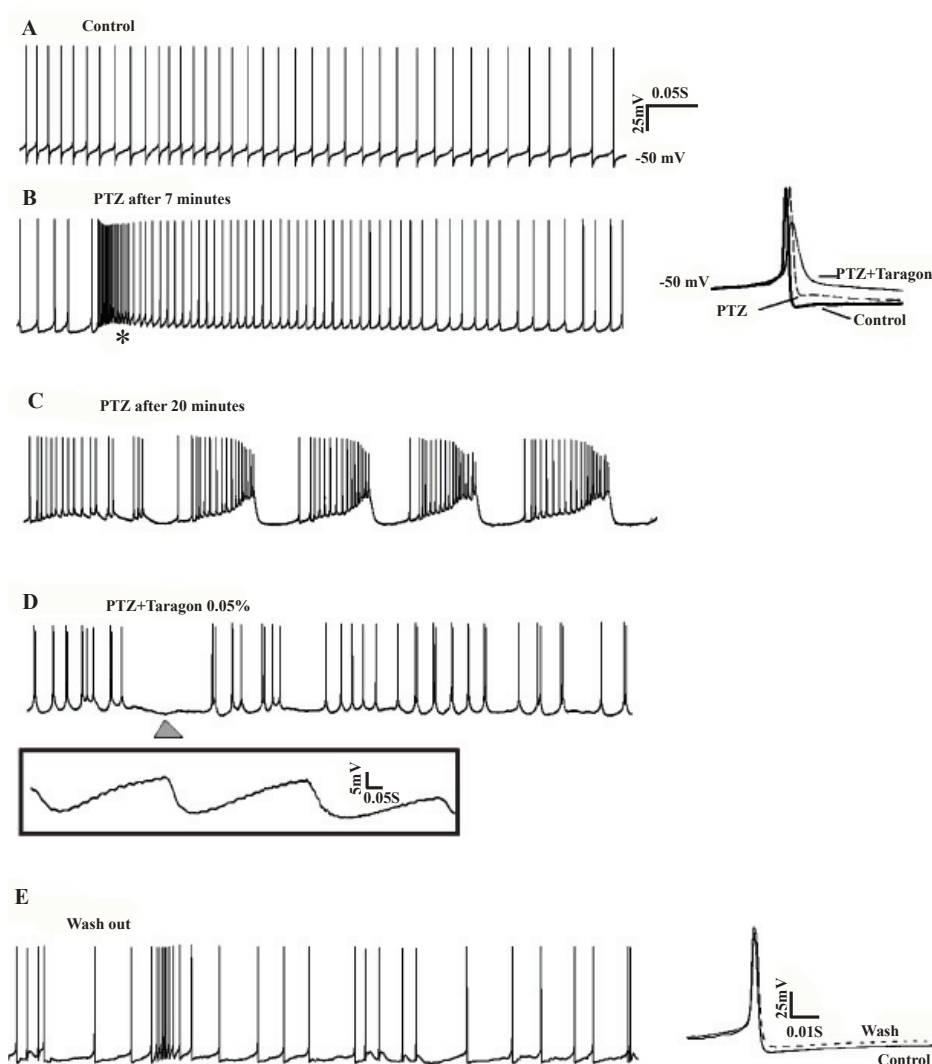
frequency ( $0.65 \pm 0.06$  Hz,  $p < 0.01$ ,  $n=20$ ) and increase in the duration of action potential ( $0.82 \pm 0.048$  mS,  $p < 0.001$ ,  $n=20$ ), but left the amplitude of action potential unchanged in compared to PTZ alone ( $53.42 \pm 3.45$  mV, Fig 1B and C,  $n=20$ ) The amplitude of AHP also decreased significantly ( $-3.36 \pm 0.2$ ,  $p < 0.001$ ,  $p < 0.01$  compared to the control and PTZ treatment, respectively, Fig 1D). To determine the reversibility of the antiepileptic effect of extract, the perfusion solution was switched from Ringer's containing PTZ and extract to normal Ringer's solution. Within 30 minutes after washing out PTZ and extract, the suppressive effects of extract (0.05%) on spontaneous activity and action potential parameters were partially reversed (Figs 1 and 2E).

Besides the assessment of the antiepileptic action of tarragon extract, its preventative effect on PTZ induced hyperexcitability was also further explored. Pretreatment of the crude ethanol extract of tarragon alone changed the neuronal firing pattern from regular tonic (Fig 3A) to an irregular mode associated with IPSPs (Fig 3B), but left the resting membrane almost unchanged. After application of tarragon extract the firing precision was assessed using the coefficient of variation (CV) of interspike intervals (ISI) of spontaneous activity ( $CV = \text{ISI S.D.} / \text{mean ISI}$ ). Application of extract altered the precision of spontaneous firing ( $CV = 2.76$  in the presence of extract vs  $1.08$  in control conditions), which showed a clear irregularity in neuronal firing. Although tarragon extract increased neuronal firing frequency, but the effect was not statistically significant (Fig 1A). Furthermore, tarragon extract led to a significant increase in the duration of action potential ( $0.51 \pm 0.019$  ms,  $p < 0.001$ ,  $n=20$ , Fig 1C) and a reduction in the amplitude of AHP ( $-8.89 \pm 0.7$  mV,  $n=20$ ,  $p < 0.001$ , Fig 2D), and the amplitude of action potential ( $79.50 \pm 2.14$ ,  $n=20$ ,  $p < 0.001$ , Fig 1B).

In an attempt to determine whether the large, long hyperpolarizations were indeed GABA mediated IPSPs or if they were mediated by intrinsic conductances, picrotoxin (100  $\mu$ M) was added to the bathing solution which contained the extract. In this condition, the administration of picrotoxin had a discernible inhibitory effect on IPSPs and neuronal excitability, since GABA receptor antagonist not only blocked the extract mediated IPSPs, but also reversed the inhibitory effect of extract on neuronal excitability (Fig 3C,  $n=5$ ). Upon application of picrotoxin, a long depolarization plateau associated with increased firing rate occurred.



**Fig 1: Effects of PTZ and tarragon on the action potential parameters of D5 neurons in *Helix aspersa*.** (A) The effects of PTZ alone, and combined treatment with PTZ and extract followed by washing out of the drugs on the firing frequency of the action potential (A), amplitude (B), duration (C) and amplitude of AHP (D). The effects of tarragon extract alone and in combination with PTZ followed by the return to normal Ringer solution on the firing frequency (A'), the amplitude (B'), the duration of action potential (C') and the amplitude of AHP (E). \* Significant difference from control, + and † denote significant difference from PTZ or extract alone, respectively. \*\*\* $p < 0.001$ ; +++ $p < 0.001$ , \*,†  $p < 0.05$ ; ††  $p < 0.01$ ; †††  $p < 0.001$  (one-way ANOVA).



**Fig 2: Effects of PTZ and *Artemisia dracuncululus* (tarragon) extract on the spontaneous firing pattern of D5 neurons.**

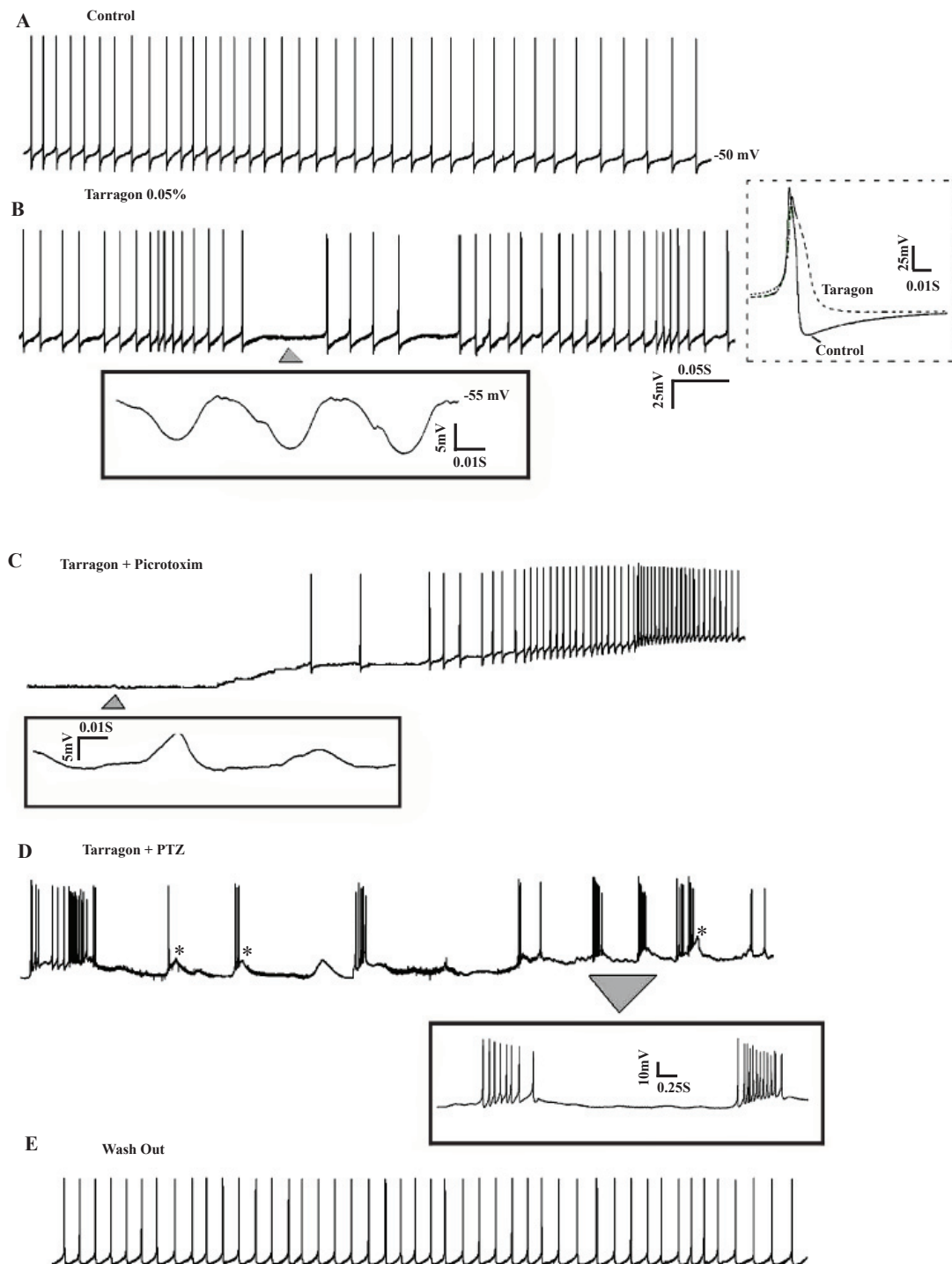
(A) Regular tonic firing activity under control conditions. Following application of PTZ, the cell resting membrane potential was slightly depolarized and PDS (asterisk) observed (B). Later the firing pattern was changed from singlet spiking to burst firing (C). Addition of extract (0.05%) in the presence of PTZ changed the firing activity to doublet spiking (D) interrupted by quiescent periods associated with IPSP (inset). The effect of the extract was almost reversible (E). All traces were recorded from the same neuron. Action potentials recorded in control, PTZ (25mM), PTZ + tarragon extract and wash out were superimposed in inset to show the effect of treatments on action potential parameters.

Following pretreatment with tarragon extract, application of PTZ produced bursts followed by brief regenerative post-spike depolarizing afterpotential (DAPs, shown in Fig. 3D by asterisks) and increased the discharge frequency ( $1.23 \pm 0.55\text{Hz}$ , Fig 1B), but later suppressed neuronal excitability and converted the action potential into subthreshold responses (data not shown). Under this condition, a significant reduction in

the amplitude of action potential in compared to the control and extract alone was observed (Fig 1B,  $p < 0.001$  and  $p < 0.001$ , respectively) and the action potentials were further broadened by PTZ treatment (Fig 1C), The mean AHP amplitude was further decreased (Fig 1D). When returned to normal Ringer's solution, the inhibitory effects of extract were almost completely reversed (Figs 1 and 3E).



## Antiepileptic Effect of Tarragon Extract



**Fig 3:** The effects of pretreatment of tarragon extract on PTZ induced hyperexcitability in D5 neurons. (A) Spontaneous tonic firing activity recorded in control conditions. (B) Tarragon extract changed the regular intrinsic firing to an irregular single spike interrupted by quiescent periods associated with IPSP (inset). (C) Application of picrotoxin (100  $\mu$ M) on tarragon treated neuron resulted in the inhibition of IPSPs associated with neuronal hyperexcitability and appearance of EPSPs (inset). (D) Addition of PTZ to the perfusing Ringer solution containing extract produced a bursting firing pattern associated with after depolarizing potential (ADP) (asterisks). Switch to normal Ringer partially recovered the normal firing pattern (E). Action potentials recorded in the control, after the application of tarragon extract and after wash out were superimposed in insets to show the effect of treatments on action potential parameters.

## Discussion

Invertebrates have often been used as experimental models for studying the effects of convulsant and anticonvulsant agents at the cellular level in order to gain a better understanding of the cellular and neurophysiological mechanisms of epileptic activity. It has been shown for instance, that convulsant drugs such as PTZ induce a potential pattern in molluscan neurons which closely resembles the epileptic activity of mammalian neurons, called PDS (11, 14, 15). In invertebrate neurons, following application of PTZ, the endogenous mechanisms are so pronounced that PDS may still be recorded in complete absence of synaptic input (10, 16).

Ion conductances underlying action potentials have been reported to participate in the generation of epileptic discharges as well as in the actions of antiepileptic drugs.  $Ca^{2+}$  and voltage-dependent  $K^+$  channels are essential in the repolarization and hyperpolarization that follows PDSs, and their mutations are substrates for neurological disorders, including epilepsy. They are the new targets for antiepileptic drugs such as retigabine (17). The knowledge of the cellular mechanisms of action of the medicinal plants with antiepileptic potential allows for design of new therapeutic approaches, possibly with fewer side effects.

A previous study has shown that the fruit essential oil obtained from the above-ground parts of tarragon blocked both clonic seizures induced by PTZ and tonic seizures induced by MES (9).

Epileptic activity can be prevented by drugs that enhance gamma amino butyric acid-type A (GABAA) receptor-mediated inhibitory neurotransmission, such as benzodiazepines and phenobarbital (18). The presence of anticonvulsant benzodiazepines in the alcoholic extract of tarragon supports the antiepileptic potential of the extract (19). Diazepam, an agonist at benzodiazepine-binding site on the GABA receptor complex, depresses the PTZ-induced bursts by exerting either depolarizing or hyperpolarizing effects and repetitive PTZ bursting activity is broken down by long inhibitory periods with large IPSPs (10). Here, similar results have been observed with extract of tarragon, which contains delorazepam and temazepam (19), against PTZ induced hyperexcitability. Tarragon extract treated neurons showed an irregular single spike firing pattern interrupted by quiescent periods associated with inhibitory post-synaptic potentials sensitive to picrotoxin. In neurons pretreated with PTZ, the amplitude of ISPCs after application of extract was small compared to the extract treatment, alone. This could be due to the partial inhibitory effect of PTZ on GABA receptor channels (20). On

the other hand, the marked increase in amplitude and duration of excitatory postsynaptic potentials (EPSPs) upon application of picrotoxin indicates that IPSPs are mediated through GABAA receptors and that GABA mediated IPSPs functionally restrict EPSPs.

Despite a direct effect of GABA receptor activation, the possibility of GABA-mediated inhibition of  $Ca^{2+}$  activated  $K^+$  channels must also be considered (21). The findings of our study showed the broadening of the action potentials and a reduction in the amplitude of action potential following AHP in the presence of the extract. We have hypothesized on the basis of our previous experimental results which have been obtained from the inhibition of AHP following apamin application, a specific SK channel blocker, (22) that reduction in the AHP amplitude after exposure to tarragon extract could be related to the inhibition of SK channels.

The neuronal excitability and fine tuning of firing patterns as well as the function and plasticity of synapses are balanced by the activity of many ion channels, including  $Ca^{2+}$ -activated  $K^+$  (SK and BK) channels (23, 24). SK channels mediate a  $Ca^{2+}$ -activated after-hyperpolarizing current, IAHP, in most neuronal cells whereas large conductance  $Ca^{2+}$ -activated  $K^+$  (BK) channels are responsible for an earlier component of the after-hyperpolarization (the fast  $Ca^{2+}$ -and voltage-dependent  $K^+$  channel currents, fAHP). In the present account, spike broadening produced by extract treatment could be related to the inhibition of  $Ca^{2+}$  and/or voltage gated  $K^+$  channels, which are responsible for the repolarization phase of action potential following AHP. Moreover, because of slower repolarization and reducing fast AHP, the rate of recovery of  $Na^+$  channel inactivation following the action potential will be slower, resulting in a decrease in the amount of recovery during the interspike interval. Thus, fewer  $Na^+$  channels will be available to depolarize the neuron in the period leading up to the next spike.

Furthermore, methyl eugenol is another component of tarragon (7), which possesses anticonvulsant, carcinogenic and genotoxic properties (25-27). Eugenol inhibits voltage-activated  $Na^+$  and  $Ca^{2+}$  channels and also voltage-gated  $K^+$  currents (28, 29).

## Conclusion

Taken collectively these data demonstrate that the crude extract of tarragon may have an antiepileptic effect against PTZ induced hyperexcitability mediated directly via activation of GABA receptors or indirectly through inhibition of calcium and/

or voltage gated potassium channels as well as through sodium channels.

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