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The fruit essential oil of *Pimpinella anisum* L. (Umblliferae) induces neuronal hyperexcitability in snail partly through attenuation of after-hyperpolarization

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ABSTRACT

Aim of the study: Many biological actions of *Pimpinella anisum* L. (Ainse), including antiepileptic activity have been demonstrated; however, there is no data concerning its precise cellular mechanisms of action. We determined whether the fruit essential oil of anise affect the bioelectrical activity of snail neurons in control condition or after pentylenetetrazol (PTZ) induced epileptic activity.
Materials and methods: Intracellular recordings were made under the current clamp condition and the effects of anise oil (0.01% or 0.05%) alone or in combination with PTZ were assessed on the firing pattern, action potential configuration and postspike potentials.
Results: Anise oil changed the firing pattern from regular tonic discharge to irregular and then to bursting in intact cells or resulted in the robustness of the burst firing and the steepness of the paroxysmal shift induced by PTZ treatment. It also significantly increased the firing frequency and decreased both the after-hyperpolarization potential (AHP) following single action potential and the post-pulse AHP.
Conclusions: Likely candidate cellular mechanisms underlying the hyperexcitability produced by anise oil include enhancement of Ca^{2+} channels activity or inhibition of voltage and/or Ca^{2+} dependent K^+ channels activity underlying AHPs. These finding indicates that a certain caution is needed when *Pimpinella anisum* is used for treating patients suffering from epilepsy.

1. Introduction

Herbal remedies are used for treating patients with various neurological disorders. Some of these products may be antiepileptic and thus possible benefit in patients suffers from epilepsy. However, the exact cellular mechanisms of action of most medicinal herbs are still not known.

Epilepsy is one of the most common neurological syndromes with a prevalence of 0.5–2%. In this study, a conventional intracellular current clamp technique was used to investigate the effect of the fruit essential oil of *Pimpinella anisum* L. on the neuronal excitability. Plant extracts and essential oils are considered nowadays as potential bioactive agents that can interfere and alter different cellular processes involved in epileptiform activity.

Pimpinella anisum L. Apiaceae (Anise) is one of the oldest known and widely used spice plants to treat a variety of ailments including epilepsy. It is native to the eastern Mediterranean and is a plant rich in volatile oils, which are employed in traditional Asian folk medicine. Several therapeutic effects including those on digestive disorders, gynecologic, and also anticonvulsant, anti-asthma and dyspnea have been described for the seeds of *Pimpinella anisum* in ancient medical books (Aboabrahim, 1970). In addition, Gülçin et al. (2003) demonstrated that water and ethanol extracts of *Pimpinella anisum* seed have potent antioxidant and antimicrobial activities.

The essential oil of anise has also been shown to possess both fungicidal and antibacterial actions (Soliman and Badeaa, 2002; Singh et al., 2002). Very recently, Al Mofleh et al. (2007) reported that aqueous suspension of anise possesses antiulcer and cytoprotective activities against chemically induced gastric lesion in rat. Furthermore, the anticonvulsant activity of the fruit essential oil of *Pimpinella anisum* was found in mice (Pourgholami et al., 1999). Recently, it has been shown that *Pimpinella anisum* oil increases glu-

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cose absorption and reduces urine output in the rat (Kreydiyyeh et al., 2003).

Although most medicinal plants appear to be relatively safe because they are natural with less side-effects, but it is deemed necessary to study in detail the cellular effect of them including *Pimpinella anisum*, which is highly used in the folk medicine. This work aimed to investigate the possible cellular mechanism(s) of the effect of the fruit essential oil of *Pimpinella anisum* on neuronal excitability and action potential characteristics in snail neurons. Using an invertebrate preparation might be an appropriate approach to facilitate the identification of a cellular or pharmacological mechanism. Moreover, the paroxysmal depolarization shifts observed in invertebrate resemble patterns of neuronal activity which can be found during epileptic seizures in vertebrates and can be called epileptiform.

2. Experimental procedures

2.1. Animal and dissection

This study was performed on the soma membrane of D5 neurons from sub-oesophageal ganglia of *Helix aspersa* (Iranian garden snail). The animals were anaesthetized by injecting them with 2 ml of 50 mM $MgCl_2$. 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 pretreatment with proteolytic enzymes. D5 neurons were visually identified by their size and color within the left parietal ganglion (Kerkut et al., 1975). The normal snail Ringer solution contained (in mM): NaCl 80, KCl 4, $CaCl_2$ 10, $MgCl_2$ 5, glucose 10, HEPES 5 (Taylor, 1987). These procedures were in accordance with the guidelines of the Institutional Animal Ethics Committee at Shahid Beheshti University of Medical Sciences.

2.2. Intracellular recording

A conventional current clamp method was applied using Axoclamp 2B amplifier (Axon Instrument, Foster City, CA, USA). The reference electrode in all experiments was silver–silver chloride wire within an agar bridge (4% agar in snail Ringer). The above set-up and recording equipment were kept in a Faraday's cage.

The electrophysiological recordings were made in real time by testing spontaneous neuronal activity, before (control), and after application of PTZ and anise oil. Data were filtered at 30 kHz, voltage records were sampled at 20 kHz and digitized online using a 16 bit A/D converter (ADInstrument Pty Ltd., Sydney, Australia) and stored for further analysis using Chart 5 and Mat-lab softwares. Further analysis was carried out by measuring the parameters of action potential, including the amplitude, and also the amplitude of after-hyperpolarization potential (AHP) (mV). The amplitude of action potential was measured as the sum of the absolute value of peak of action potential and the peak of AHP. The amplitude of AHP was measured from the resting membrane potential to the end of the spike repolarization.

2.3. Plant material and drugs

Fruits of anise were obtained from a local market. The plant was authenticated by M. Kamalinejad (Department of Pharmacognosy, Faculty of Pharmacy, Shahid Beheshti University, Medical Campus, Tehran, Iran) and a voucher specimen coded P-544 has been deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Shahid Beheshti University (Medical Campus), Tehran, Iran. The fruits were processed by steam distillation over a period of 4 h in all glass apparatus, to obtain the essential oil with 2% yield. Preliminary experiments were performed to establish the minimal anise oil concentrations (0.01% and 0.05%) required to influence the neuronal excitability. Pentylenetetrazol (PTZ) was applied (25 mM) into the bathing solution. The pH of solutions was adjusted to 7.6 with Trizma base (Sigma). Each solution was superfused into the experimental chamber at a rate of approximately 2.5 ml/min.

2.4. Statistical analysis

Numerical results are given as mean ± S.E.M., with n being the number of cells on which the measurement was done. Significant differences between the groups were evaluated using a Student's t-test or one-way ANOVA and $P < 0.05$ was considered to be significant.

3. Results

In the first part of this study, the antiepileptic potential of the essential oil of *Pimpinella anisum* was examined on the PTZ induced epileptiform activity and in the second part of the study, the possible preventative potential of anise oil was investigated. Conventional intracellular recordings, in current clamp mode, were obtained from 21 D5 neurons from the left parietal ganglion of *Helix aspersa*. Experiments were carried out in the PTZ pretreated or PTZ post-treated conditions to analyze the effects of the fruit essential oil of anise on the spontaneous bioelectrical activity and action potentials configuration of D5 cells. Neurons selected for drug application had resting membrane potentials of at least −45 mV and action potential amplitudes exceeding 50 mV.

D5 cells in normal Ringer fired tonically (Figs. 1A and 2A, control) and had a mean resting membrane potential (RMP) of -46.71 ± 2.2 mV and exhibited spontaneous regularly spaced action potentials with a mean frequency of 0.73 ± 0.14 Hz (Figs. 1D, E and 2D, E, control) and amplitude of 93.62 ± 3.4 mV. Single action potentials were followed by AHP with mean amplitude of -10.6 ± 1 (Figs. 1B, C and 2B, C).

3.1. Effects of the essential oil of Pimpinella anisum on PTZ-induced epileptic activity

To investigate the effect of *Pimpinella anisum* on PTZ-induced epileptiform activity, the fruit essential oil of anise (0.01% and 0.05%) was applied extracellularly. In current clamp condition, PTZ treated neurons showed increased spontaneous activity and a typical change of the steepness of paroxysmal depolarization was appeared (Fig. 1A). Furthermore, PTZ application led to a significant reduction in the AHP amplitude (Fig. 1B and C) and an increase in the firing frequency (Fig. 1D and E). When PTZ treated neurons were exposed to the Ringer solution containing PTZ + anise (0.01 or 0.05), a robust PDS was produced and potentiation of the epileptic activity was occurred (Fig. 1A; PTZ). Combined treatment with PTZ and anise (0.01%) caused a significant reduction of the peak amplitude of AHP following action potentials ($P < 0.001$ and $P < 0.01$, Fig. 1B, compare to control and PTZ treatment alone, respectively) or complete disappearance (at concentration of 0.05% anise, Fig. 1C) of AHP. In addition, the firing frequency of PTZ treated neurons was significantly increased ($P < 0.001$, compare to control and $P < 0.01$ compare to PTZ alone) or decreased ($P < 0.001$ compare to control and $P < 0.01$ compare to PTZ alone), when the recording solution switched to Ringer containing PTZ + anise 0.0.1% or 0.05%, respectively (Fig. 1D and E).

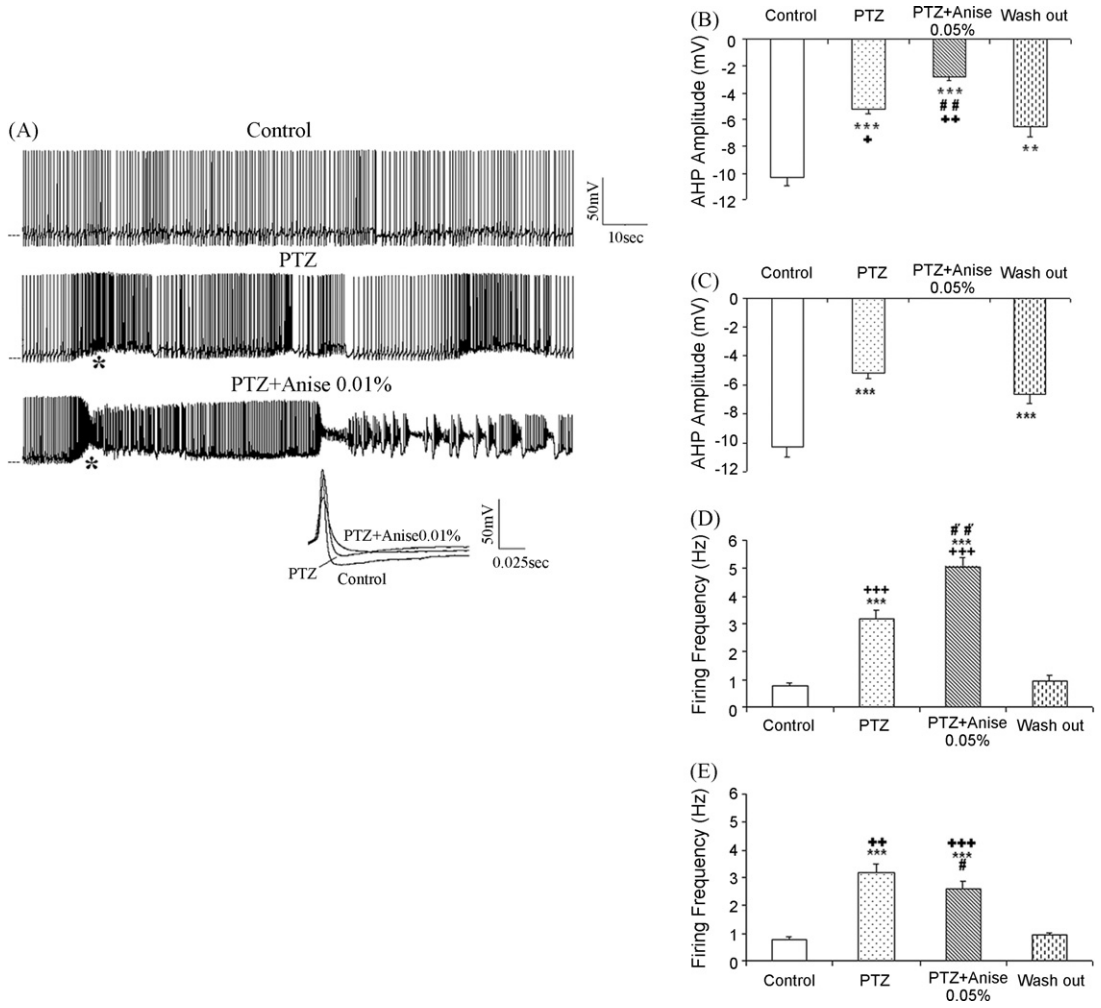

Fig. 1. Effect of anise essential oil on PTZ induced epileptiform, AHP amplitude and firing frequency of D5 neurons. (A) D5 neuronal firing pattern in control, 10 min after PTZ (25 mM) application, which was associated with bursting and PDS (asterisks) and following combined application of PTZ plus anise (0.01%). The horizontal dashed lines at the left side of control, PTZ (25 mM) and anise + PTZ traces indicate the membrane potential at −45 mV. Inset shows superimposed recordings of action potentials from a D5 neuron before (control) and after application of PTZ and anise oil (0.01%) + PTZ. Histograms represent the mean amplitude of AHP (B and C) and firing frequency (D and E) of 8 neurons in control, 10 min after PTZ and 15 min after combined treatment with PTZ + anise at concentration of 0.01% (B and D) or 0.05% (C and E). The effects of anise (0.01–0.05%) + PTZ were partially reversible, when perfusion Ringer solution containing PTZ plus anise was replaced by normal Ringer solution (wash out). $^{*,\#,+}$Significant differences from control, PTZ and combined PTZ and anise treatments, respectively. $^{***,+++}P < 0.001$, $^{*,+}P < 0.05$, $^{++,\#\#}P < 0.01$ as compared by one-way ANOVA test.

3.2. Effects of pretreatment with fruit essential oil of Pimpinella anisum on the induction of epileptic activity by PTZ

Application of the fruit essential oil of anise at concentration of 0.01% or 0.05% revealed that exposure to low concentrations of anise considerably change the neuronal firing behavior (Fig. 2A). Anise treated neurons exhibited a firing pattern consisted of a period of tonic discharge with increase in firing frequency (Fig. 2D and E), followed by a paroxysmal depolarization shift (shown as asterisk in Fig. 2A, anise 0.01%) and then a period of bursting activity. As a measure of regularity of firing, the coefficient of variation (CV) of interspike interval (ISI) between spikes collected during 5 s recording period. The mean and standard deviation of ISIs were calculated and the coefficient of variation was derived from the ratio of S.D.: mean ISI. Clear and regularly spaced peaks of action potentials observed for tonically firing D5 cells in control condition was associated with low CV (0.26). Anise treatment increased the coefficient of variation to 0.5 (about 90% increases), which indicates that anise increases the irregularity of firing and makes the cell bursty. Furthermore, neither the RMP nor the amplitude and duration of action potential were significantly affected by anise treatment alone (data not shown). While the amplitude of AHP recorded in the presence of anise (0.01% and 0.05%) were significantly reduced within 15 min of exposure (Fig. 2B and C).

In order to examine the effect of pretreatment of anise on the induction of epileptic activity, PTZ (25 mM) was added to the Ringer containing anise oil. After exposure of D5 neurons treated with anise to PTZ, the spontaneous activity of these neurons was increased and a robust PDS followed by burst firing was induced and then a sustained depolarization potential was established (Fig. 2A; anise + PTZ). Moreover, the firing frequency of anise treated neurons was significantly increased (Fig. 2D and E).

The effect of combined treatment with anise (0.01%) and PTZ on the amplitude of AHP was partially reversible, but on the firing frequency was irreversible even after 45 min of washing. While at concentration of 0.05% neither the amplitude of AHP nor the firing frequency returned to control values (Fig. 2B–E).

In control condition, depolarizing current injections (1–5 nA for 500 ms) was associated with increasing in the firing rate of action potentials and followed by a post-pulse AHP (data not shown).

Anise treatment also resulted in a reduction in the amplitude of post-pulse AHP (Fig. 3).

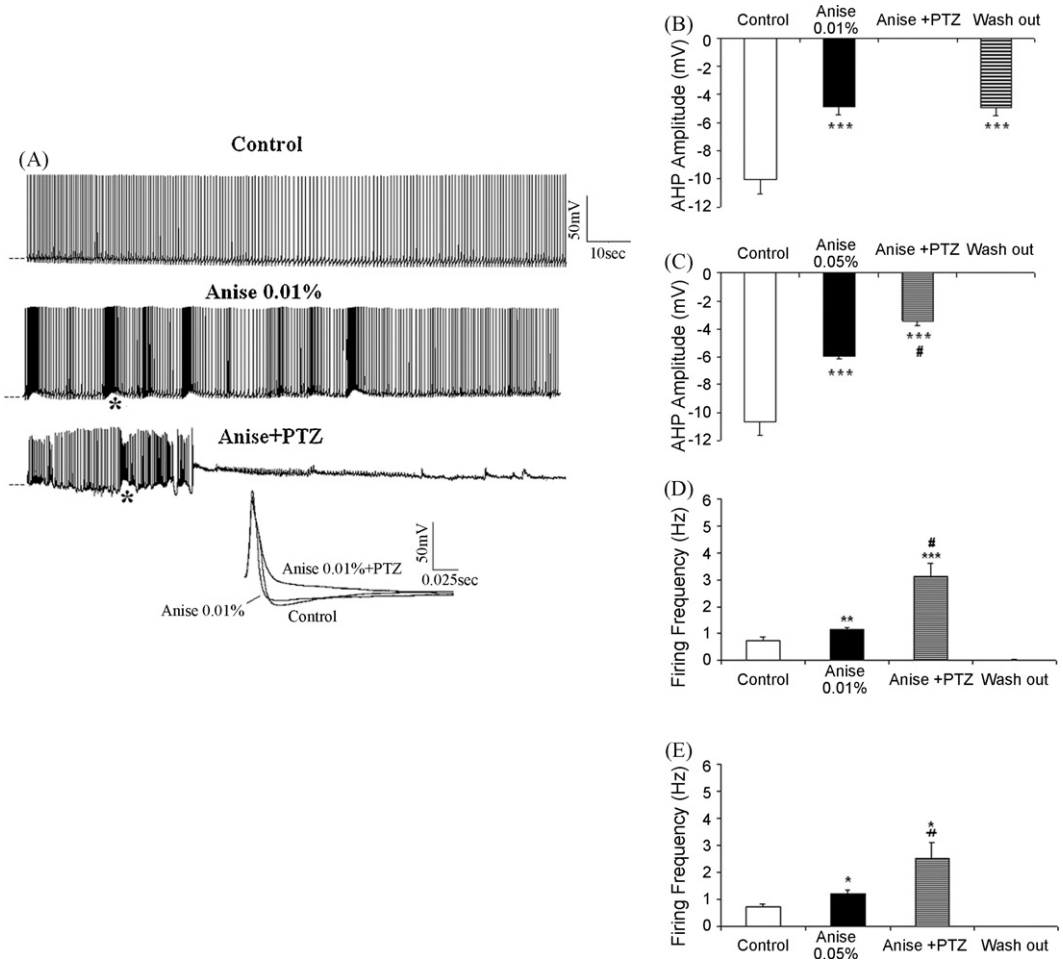

Fig. 2. Effect of pretreatment with anise oil (0.01% or 0.05%) on the induction of epileptic activity by PTZ, amplitude of AHP and firing frequency of D5 neurons. (A) Autonomous activity of a representative neuron under the control condition; 15 min after application of anise (0.01%) and 10 min after combined application of PTZ and anise (0.01). Asterisks show paroxysmal depolarization shift. Inset shows superimposed action potentials recorded in control, after exposure to anise 0.01% and anise + PTZ. All traces were recorded from the same neuron and action potentials were truncated in inset. Histograms represent the mean amplitude of AHP (B and C) and firing frequency (D and E) of 8 neurons in control; 15 min after anise 0.01% (B and D) and 0.05% (C and E); 10 min after combine treatment with anise (0.01% or 0.05%) + PTZ and washing out the anise oil and PTZ with replacing the extracellular solution containing drugs with normal Ringer. $^{*,\#,+}$Significant differences from anise treatment alone and wash out, respectively. $^{*,\#}P<0.05$, $^{**,++}P<0.01$, $^{***}P<0.001$, as compared by one-way ANOVA test.

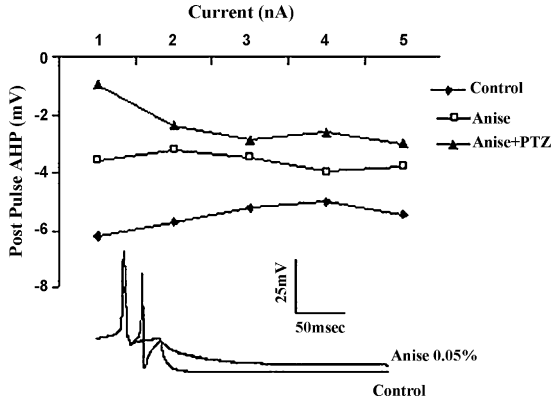

Fig. 3. Effect of anise on the post-train AHP of D5 neurons. Post-train AHP was reliably reduced in the magnitude by application of anise (0.05%) prior to PTZ treatment. Subsequent application of PTZ in the presence of anise further decreased the amplitude of post train AHP. Inset shows superimposed truncated evoked responses to a depolarizing current (3 nA, 500 ms) in control condition and after application of anise (0.05%). Anise reduced the amplitude of post-train AHP.

At higher concentrations (>1%) anise oil switched the firing pattern from regular tonic to bursting and there was progressive epileptiform activity manifested in the form of Na^+ action potentials and doublets of Ca^{2+} spikes (Fig. 4).

4. Discussion

Aromatic spice plants have been used traditionally as food and for medicinal purposes in the therapy of some diseases for a long time in the world. Essential oils in these plants are used extensively in medicine and in the food and cosmetic industries. As an aromatic plant, anise (*Pimpinella anisum* L.) is an annual herb indigenous to Iran, India, Turkey, Mexico, Chile and many other warm regions in the world.

The present findings described the cellular effects of the fruit essential oil of anise on the neuronal excitability and action potential characteristics. It increased the irregularity of firing pattern and decreased temporal precision of action potential, thereby caused neuronal hyperexcitability leading to burst of action potentials and epileptiform activity. It also potentiated the PTZ-induced neuronal hyperexcitability and at higher concentration (5%) produced a bursting pattern consists of mainly Na^+ and doublets of Ca^{2+}

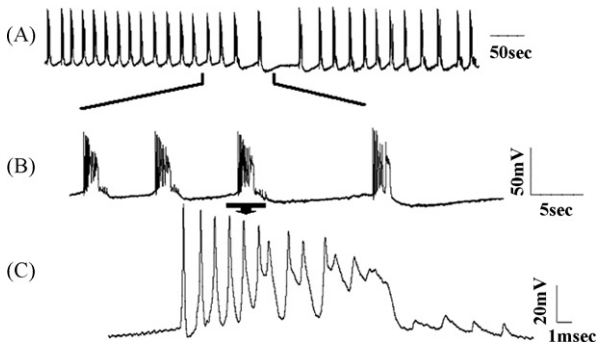

Fig. 4. High concentration of anise oil converted the tonic regular firing behavior of D5 neuron to a bursting mode. Application of higher concentration of *Pimpinella anisum* oil (5%) converted the regular neuronal firing observed in control conditions (see Figs. 1 and 2; control) to a bursting mode (A and B). Expanded picture of an individual burst showing that each burst starts with Na^+ action potentials, followed by several doublets and broader Ca^{2+} spikes (C).

action potentials. The hyperexcitability induced by anise essential oil could be mediated through activation of Ca^{2+} channels or inhibition of voltage and/or calcium dependent K^+ channels. The increased neuronal activity after exposure to anise oil is in line with the observed decreased in AHP amplitude. Our previous work revealed that an apamin sensitive Ca^{2+} activated potassium channels contribute to the AHP in snail neurons; so that apamin, an specific blocker of small conductance K_{Ca} channels, eliminated a major component of AHP and increased the frequency of Ca^{2+} spikes (Vatanparast et al., 2006a). The AHP that follows action potentials is a key determinant of cellular excitability and an important intrinsic negative feedback mechanism firing pattern excitability (Aizenman and Linden, 1999; Bevan and Wilson, 1999). Alterations in the amplitude and duration of AHP have been shown previously to influence neuronal excitability in many different neurons (Yarom et al., 1985; Goh and Pennefather, 1987; Sah, 1996; Wu et al., 2004). A decrease in the amplitude of AHP increases the neuronal excitability, respectively (Kawai and Watanabe, 1986; Madison and Nicoll, 1986).

In invertebrate neurons, the action potential is followed by an after-hyperpolarization, produced by the voltage and/or Ca^{2+}-dependent potassium channels activated during the spike, which transiently hyperpolarizes the membrane and then deactivate slowly. Based on current clamp condition we found that *Pimpinella anisum* essential oil led to a decrease in the amplitude of AHPs. In snail neurons, spike repolarization and AHP are determined by a set of potassium channels which underlie fast and delayed K^+ outward currents (Thompson, 1977; Solntseva, 1995; Bal et al., 2000, 2001; Sakakibara et al., 2005), and also two classes of Ca^{2+} activated K^+ channels; large conductance Ca^{2+} activated K^+ channels (BK channels) and small conductance Ca^{2+} activated K^+ channels (SK channels) (Hermann and Erxleben, 1987; Gola et al., 1990; Crest and Gola, 1993). In many neurons, Ca^{2+} influx through voltage dependent Ca^{2+} channels and consequent activation of Ca^{2+} dependent potassium channels is a major determinant of AHP amplitude and duration.

Previous chemical studies reported that the *Pimpinella anisum* seeds (fruits) contain 90% of anothole (Chandler and Hawkes, 1984; Pourgholami et al., 1999). The other major compounds are estragole (Zargari, 1989), eugenol (Monod and Dortan, 1950), methylchavicol, anisaldehyde (Wagner et al., 1984), terpene hydrocarbons (Kartnig et al., 1975), polyenes and polyacetylenes (Schulte et al., 1970). Among these compounds, it was reported that anethole at lower concentrations activates voltage-dependent Ca^{2+} channels (Soares et al., 2007). There is considerable evidence available concerning the potential role of voltage dependent calcium channels in epileptogenesis (Jones, 2002). Therefore, a possible explanation of anise induced bursting activity could be an increase in the Ca^{2+} influx through activation of voltage gated Ca^{2+} channels (Faizi et al., 2003), which needs to be further elucidate using voltage clamp technique. The depolarizing effect of some anticonvulsant drugs such as diazepam has already been described in snail neurons so that following application of diazepam an increase in the firing rate and potentiation of PTZ induced bursting activity has been observed (Faugier-Grimaud, 1978). Pourgholami et al. (1999) reported the anticonvulsant action of the fruit essential oil of *Pimpinella anisum* in mice. Similar to the reported effects of diazepam as an anticonvulsant agent on PTZ-induced epileptiform activity in snail neurons, in the present work was shown that essential oil of *Pimpinella anisum* increases the rate and irregularity of firing, therefore, the anticonvulsant effect of anise oil observed in mice possibly could be exerted in the same way as diazepam. In conclusion, on the basis of the present data in combination with the previous work on the blockage of calcium activated potassium channels following application of apamin or intracellular injection of BAPTA (Vatanparast et al., 2006b) in snail neurons, it can be suggested that the fruit essential oil of anise induces hyperexcitability through inhibition of K_{Ca} channels. Therefore, due to potential capacity of anise to induce neuronal hyperexcitability, when it is used for treating patients suffer from epilepsy, a certain caution is needed.

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References

Aboabrahim, Z., 1970. Zakhirah Kharazmshahi, vol. 2. National Works Publications, Tehran, p. 141.
Aizenman, C.D., Linden, D.J., 1999. Regulation of the rebound depolarization and spontaneous firing patterns of deep nuclear neurons in slices of rat cerebellum. Journal of Neurophysiology 82, 1697–1709.
Al Mofleh, I.A., Alhaider, A.A., Mossa, J.S., Al-Soohaibani, M.O., Rafatullah, S., 2007. Aqueous suspension of anise "*Pimpinella anisum*" protects rats against chemically induced gastric ulcers. World Journal of Gastroenterology 13, 1112–1118.
Bal, R., Janahmadi, M., Green, G.G.R., Sanders, D.J., 2000. Effect of calcium channel blockers on transient outward current of F76 and D1 neuronal soma membrane in the subesophageal ganglion of *Helix aspersa*. Journal of Membrane Biology 173, 179–185.
Bal, R., Janahmadi, M., Green, G.G., Sanders, D.J., 2001. Two kinds of transient outward currents, I_A and I_{Adepol}, in F76 and D1 soma membranes of the subesophageal ganglia of *Helix aspersa*. Journal of Membrane Biology 179, 71–78.
Bevan, M.D., Wilson, C.J., 1999. Mechanisms underlying spontaneous oscillation and rhythmic firing in rat subthalamic neurons. Journal of Neuroscience 19, 7617–7628.
Chandler, R.F., Hawkes, D., 1984. Aniseed: spice, flavour, drug. Journal of Canadian Pharmacology 117, 28–29.
Crest, M., Gola, M., 1993. Large conductance Ca^{2+}-activated K^+ channels are involved in both spike shaping and firing regulation in Helix neurones. Journal of Physiology 465, 265–287.
Faizi, M., Janahmadi, M., Mahmoudian, M., 2003. The effect of mebudipine and dibudipine, two new Ca^{2+} channel blockers, in comparison with nifedipine on Ca^{2+} spikes of F1 neuronal soma membrane in *Helix aspersa*. Acta Physiologica Hungarica 90, 243–254.
Faugier-Grimaud, S., 1978. Action of anticonvoulsants on pentylenetetrazol-induced epileptiform activity on invertebrate neurones (*Helix aspersa*). Neuropharmacology 17, 905–918.
Goh, J.W., Pennefather, P.S., 1987. Pharmacological and physiological properties of the afterhyperpolarization current of bullfrog ganglion neurones. Journal of Physiology 394, 315–330.
Gola, M.C., Ducreux, C., Chagneux, H., 1990. Ca^{2+} activated K^+ current involvement in neuronal function revealed by in situ single channel analysis in Helix neurones. Journal of Physiology 420, 73–109.
Gülçin, I., Oktay, M., Kireçci, E., Küfrevioglu, O.I., 2003. Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. Food Chemistry 83, 371–382.

Hermann, A., Erxleben, C., 1987. Charybdotoxin selectively blocks small Ca^{2+} activated K^+ channels in Aplysia neurones. The Journal of General Physiology 90, 27–47.

Jones, O.T., 2002. Ca^{2+} channels and epilepsy. European Journal of Pharmacology 447, 211–225.

Kawai, T., Watanabe, M., 1986. Blockage of Ca^{2+} activated K^+ conductance by apamin in rat sympathetic neurones. British Journal of Pharmacology 87, 225–232.

Kartnig, T., Moeckel, H., Mauns, B., 1975. Occurrence of coumarins and sterols in tissue cultures of roots of *Anethum graveolens* and *Pimpinella anisum*. Planta Medica 27, 1–4.

Kerkut, G.A., Lambert, J.D.C., Gayton, R.J., Locker, J.E., Walker, R.J., 1975. Mapping of nerve cells in the sub-oesophageal ganglia of *Helix aspersa*. Comparative Biochemistry and Physiology (A) 50, 1–25.

Kreydiyyeh, S.I., Usta, J., Knio, K., Markossian, S., Dagher, S., 2003. Aniseed oil increases glucose absorption and reduces urine output in the rat. Life Science 74, 663–673.

Madison, D.V., Nicoll, R.A., 1986. Cyclic adenosine 30,50 monophosphate mediates beta-receptor actions of noradrenaline in rat hippocampal pyramidal cells. Journal of Physiology 372, 245–259.

Monod, C., Dortan, D., 1950. Eugenol in anise oil. Chemical Abstracts 45, 3124.

Pourgholami, M.H., Majzoob, S., Javadi, M., Kamalinejad, M., Fanaee, G.H.R., 1999. The fruit essential oil of *Pimpinella anisum* exerts anticonvulsant effects in mice. Journal of Ethnopharmacology 66, 211–215.

Sah, P., 1996. Ca^{2+}-activated K^+ currents in neurones: types, physiological roles and modulation. TINS 19, 150–154.

Sakakibara, M., Okuda, F., Nomura, K., Watanabe, K., Meng, H., Horikoshi, T., Lukowiak, K., 2005. Potassium currents in isolated statocyst neurones and RPeD1 in the pond snail *Lymnaea stagnalis*. Journal of Neurophysiology 94, 3884–3892.

Schulte, K.E., Rucker, G., Backe, W., 1970. Polyacetylenes from *Pimpinella anisum* species. Archive Der Pharmazie 303, 912–919.

Singh, G., Kapoor, I.P.S., Pandey, S.K., Singh, U.K., Singh, R.K., 2002. Studies of essential oils. Part 10. Antibacterial activity of volatile oils of some spices. Phytotherapy Research 16, 680–682.

Soares, P.M.G., Lima, R.F., de Freitas Pires, A., Souza, E.P., Assreuy, A.M.S., Criddle, D.N., 2007. Effects of anethole and structural analogues on the contractility of rat isolated aorta: involvement of voltage-dependent Ca^{2+}-channels. Life Science 81, 1085–1093.

Soliman, K.M., Badeaa, R.I., 2002. Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. Food Chemical Toxicology 40, 1669–1675.

Solntseva, E.I., 1995. Properties of slow early potassium current in neurones of snail *Helix pomatia*. General Pharmacology 26, 1719–1726.

Thompson, S.H., 1977. Three pharmacologically distinct potassium channels in molluscan neurones. Journal of Physiology 265, 465–488.

Taylor, P.S., 1987. Selectivity and patch measurements of A-current channels in *Helix aspersa* neurones. Journal of Physiology 388, 437–447.

Vatanparast, J., Janahmadi, M., Asgari, A.R., Sepehri, H., Haeri-Rohani, A., 2006a. Paraoxon suppresses Ca^{2+} spike and after-hyperpolarization in snail neurons: relevance to hyperexcitability induction. Brain Research 1083, 110–117.

Vatanparast, J., Janahmadi, M., Asgari, A.R., 2006b. The functional consequences of paraoxon exposure in central neurones of lands snail, *Caucasotacheea atrolabiata*, are partly mediated through modulation of Ca^{2+} and Ca^{2+} activated K^+ channels. Comparative Biochemistry and Physiology Part C 143, 464–472.

Wagner, H., Bladt, S., Zgainski, E.M., 1984. Plant Drug Analysis. Springer–Verlag, New York.

Wu, W.W., Chan, C.S., Disterhoft, J.F., 2004. Slow afterhyperpolarization governs the development of NMDA receptor-dependent afterhyperpolarization in CA1 pyramidal neurons during synaptic stimulation. Journal of Neurophysiology 92, 2346–2356.

Yarom, Y., Sugimori, M., Llinas, R., 1985. Ionic currents and firing patterns of mammalian vagal motoneurons in vitro. Neuroscience 16, 719–737.

Zargari, A., 1989. Medicinal Plants, 2. Tehran University, Tehran.