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Short Communication

Anticonvulsant effects of squill oxymel (a traditional formulation) in mice

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ABSTRACT

Introduction: This study evaluated the anticonvulsant activity of the add-on *Drimia maritima* (squill) oxymel, used traditionally in the treatment of convulsion, in animal model.

Methods: Albino mice pretreated with squill oxymel in different doses of 50, 100, 200 and 400mg/kg by oral gavage, 15min prior to injection of pentylenetetrazole (PTZ). Animals pretreated with flumazenil to determine the mechanism of anticonvulsant action. The total flavonoid content of squill oxymel was also determined.

Results: Squill oxymel prolonged the onset of seizures and decreased the duration of seizures compared to control group. Diazepam used as a reference drug for its anticonvulsive effects, showed complete inhibition of seizure. This study revealed that squill oxymel has significant anticonvulsant effect in PTZ-induced seizures in mice and these effects may be related to its effect on benzodiazepines' receptors on GABA complex.

Conclusion: These results confirmed the traditional use of squill oxymel in Iranian traditional medicine for treatment of epilepsy. The clarification of mechanisms involved needs further studies.

Keywords:
Drimia maritime
Squill
Oxymel
Anticonvulsant
Pentylenetetrazole

Introduction

Epilepsy is one of the most complicated neurological disorders that described as recurrent spontaneous seizures (Motevalian et al., 2017). It is estimated about 65 million people suffering from epilepsy around the world (Ngugi et al., 2010). About 70% of epileptic patients are able to treat with the various kinds of modern anticonvulsant drugs that can prevent or decrease the number of seizure attacks (Thurman et al., 2011). Use of multiple anticonvulsant drugs may induce some adverse effects such as inhibition or induction of liver enzymes and drug interaction. On the other hand, 30% of epileptic patients are still suffering from uncontrollable seizures even with multiple drugs (Johannessen Landmark and Patsalos, 2010; Kirmani et

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al., 2014). There are some herbs or herbal products which have been recommended to treat seizure and epilepsy. *Drimia maritima (L.) Stearn* (Asparagaceae) commonly known as squill, is one of the herbs which mentioned in Iranian traditional medicine (ITM) for epilepsy (Ardakani, 2018). Genus Drimia are distributed in Mediterranean area, Africa and India. The most important medicinal part of squill is mostly bulb (Zargari, 1996). The main secondary metabolites are cardiac glycosides specially bufadenolides, which are responsible for its cardiac effects. Other compounds of *D. maritima* includes alkaloids, flavonoids, tannins, sterols and terpenoids (Belhaddad et al., 2018). The main properties of squill considered in ITM are nervous ailments such as amnesia, epilepsy, melancholy and other disorders like jaundice, dropsy, cough and respiratory ailments (Avicenna, 2008; Bozorgi et al., 2017; Chamberlain and Levy, 1937; Stannard, 1974). In addition to cardiovascular properties, other biological effects such as antitumor, antioxidant and insecticidal activity have been reported from this plant (Nejatbakhsh et al., 2017). According to toxic properties of squill bulb, ancient text recommended the use of squill bulb in the form of oxymel preparation to treat epilepsy. It has been shown that processing squill with vinegar can reduce possible side effects of bufadienolids of squill (Bozorgi et al., 2017). Following our previous studies on the anticonvulsant effects of herbal medicine in ITM (Mehrzadi et al., 2016; Motevalian et al., 2017), in the present study anticonvulsant activity of the oxymel of *Drimia maritima* (a traditional formulation) has been investigated in animal model of convulsion.

Methods and materials

Plant material

The oxymel of *D. maritima* bulb was purchased from Barij essence pharmaceutical company (Kashan, Iran) which containing squill bulb, vinegar and honey and was assessed by Razi Drug Research Center in Iran University of Medical Sciences, Tehran, Iran.

Animals

Thirty male NMRI mice weighing 30±4g were provided from Pasteur Institute (Tehran, Iran). The Animals were kept in the groups of 5 and housed in Polycarbonate cages under standard laboratory conditions (temperature 23±2°C with 12h light/dark cycles) and kept in the Vivarium of Iran University of Medical Sciences. The animals acclimatized at least a day before experiment with free access to standard diet and water. The whole process done in 2 weeks. Each mouse used only once and all the procedures recorded by camera (TSCO Webcam TW 1200K, USA) and investigated carefully in order to make the final results more reliable. In addition, efforts have made to minimize the animal's suffering and reduce the number of used animals. The experimental procedure of this study was approved by the Ethical Committee of Iran University of Medical Sciences (Ethical Code: IR.IUMS.FMD.REC.1399.138) and all the procedures were conducted in alignment with the National Institutes of Health Guidelines in terms of Care and Use of Laboratory Animals (NIH Publication No. 85–23, revised 1996).

Drugs and chemicals

Pentylenetetrazole (PTZ; 60mg/kg, Sigma, St. Luis, MO, USA) and diazepam (DZP; 1mg/kg, Chemidarou, Iran), were provided from commercial sources. All drugs were dissolved in normal saline and prepared freshly before intraperitoneal administration.

Phytochemical screening

The total phenolic content of the squill oxymel was calculated by colorimetric method using the Folin-Ciocalteu reagent (Haile and Kang, 2019). For the preparation of linear calibration curve, 1ml of gallic acid standards at different concentrations (75, 100, 150 and 200 µg/ml hydro-ethanolic (50:50) were mixed with 5ml Folin–Ciocalteu reagent (diluted ten-fold) and 3ml sodium carbonate (2% w/v). The absorption was read after 2h at 760nm and the calibration curve was drawn. The same procedure was carried out on squill oxymel (1mg/ml) and the absorbance was measured at 760nm for the determination of plant phenolics. All procedures were repeated in triplicate (Shahidi and Ho, 2007).

Total flavonoid content

The total flavonoid content of squill oxymel was determined according to aluminum chloride reagent. To prepare the catechin calibration curve. Briefly, 1ml of catechin as standard or other samples (previously dissolved in 90% ethanol), were thoroughly mixed with 0.2ml of a 5% $NaNO_2$. After 5min, 0.3ml of 3% $AlCl_3$

solution and 2ml of 2M NaOH were added. The absorbance was measured after 30min at 510nm (Greenberg, 1981).

Anticonvulsant activity

Convulsion was induced by PTZ (60mg/kg, IP) in the mice (Mehrzadi et al., 2016). Thirty mice were randomly divided into six groups (n=5 in each). Control group received normal saline (0.9%, IP) as group 1. The positive control group (group 2) received diazepam (1mg/kg, IP) 15min before the administration of PTZ (Mehrzadi et al., 2015). Groups 3-6 of mice were given different doses of *Drimia maritima* oxymel (50, 100, 200, 400mg/kg) by oral gavage, 15min before the injection of chemo-convulsant to animals. Each animal was held and observed inside an individual polycarbonate cage carefully for 30min and the seizure parameters were recorded (Rashidian et al., 2016). The period of time before the convulsion onset (latency), duration of clonic convulsion and the percentage of animal's mortality and protection were recorded (Rashidian et al., 2017).

Statistical analysis

The results of study are reported as (mean± standard error of the mean or mean ± standard diviation). The statistical analysis was carried on using one-way analysis of variance (ANOVA) by Graph Prism 7.0 software. Tukey's post-test was used. All the group differences were calculated and for all groups, differences with values of $P<0.05$ were regarded as significant.

Results

Total phenolic and flavonoid contents

The total phenolic content of squill oxymel was expressed as mean ± standard deviations of µg of gallic acid (GAE) equivalents/ mg extract. The total flavonoid content of squill oxymel was calculated on the basis of a linear calibration curve obtained using catechin and expressed as µg catechin equivalents/mg of sample (Table 1).

Anticonvulsant activity

In the PTZ-induced anticonvulsant activity of squill oxymel, a prolonged time of onset of seizure was observed with higher doses of 100 and 200mg/kg ($P<0.05$). Moreover, squill oxymel at the doses of 100, 200 and 400 mg/kg ($P<0.05$) decreased the duration of seizure in comparison to normal saline control group. Squill oxymel showed its impact on reducing the mortality rate of animals in a dose-dependent manner. Mortality pro-

TABLE 1: Total phenolic and flavonoid contents of squill oxymel

	Calibration curves	R^2	Phenolics/flavonoid contents (µg/mg)*
Phenolic content determination	y=0.00099x+0.06123	0.9993	0.284± 0.03
Flavonoid content determination	y=0.002x+0.0082	0.9995	0.048 ± 0.003

*Presented as µg of gallic acid and catechin equivalents in/ mg of sample. Values are expressed as mean±SEM (n=3)

TABLE 2: The effects of squill oxymel on PTZ-induced Convulsion in Mice

Treatment (Dose)	Onset (s)	Duration (s)	Mortality (%)
Normal saline + PTZ	42 ± 2.8	35.9 ± 2.8	20
Diazepam (1mg/kg) + PTZ	------	------	0
SO (50mg/kg) + PTZ	68.33 ± 16.3	30.76 ± 6.4	20
SO (100mg/kg) + PTZ	94.75 ± 21.7*	22.1 ± 7.7*	0
SO (200mg/kg) + PTZ	100.5 ± 16.2*	18.5 ± 1.5*	0
SO (400mg/kg) + PTZ	77.8 ± 20.9	19.92 ± 5.8*	0

*$P<0.05$ compared with the normal saline group (negative control). PTZ: Pentylenetetrazole; SO: squill oxymel

tection (%) in diazepam and squill oxymel (doses 100, 200 and 400mg/kg) groups were 100%, however it is much less in control group and in squill oxymel (50mg/kg) group (about 80%). In addition, diazepam as a reference drug with anticonvulsant activity caused full protection against seizure (Table 2).

Discussion

Epilepsy is one of the most common neurological disorders which affect about 50 million people worldwide (Moshi et al., 2005). There are some herbs or herbal preparations which have been recommended to treat seizure and epilepsy in ITM. The *D. maritima* (Asparagaceae) commonly known as squill, is one of the herbs which mentioned in ITM in the form of oxymel for treatment of epilepsy. In present study, anticonvulsent activity of squill oxymel was tested in animal model of convulsion. The results of the study showed that different doses of squill oxymel can decrease the duration period of seizure in comparison with negative control group. Also, the oxymel reduced the mortality rate of animals in a dose-dependent manner.

The bulb of *D. maritima* is toxic for mice and not recommended to use without modification in human. In ITM, the vinegar and oxymel of squill have been suggested for epileptic patients (Aghili Khorasani et al., 2011). There are some experimental, clinical and phytochemical studies on biological activities and chemical constituents of squill bulb. In a study by Nejatbakhsh et al. (2017), squill oxymel showed significant efficacy in patients with moderate to severe persistent asthma as add-on therapy. The extract of *D. maritima* have been reported to show strong antioxidant activity (Mammadov et al., 2010). It showed positive inotropic and hypotensive effect in rabbits (Dizaye and Hamad, 2010). There is also evidence of squill's antibacterial (Belhaddad et al., 2018) and anticancer activities (Obeidat and Sharab, 2018).

Bufadenolides have been identified as the main constituents in the genus of Drimia. Some other constituents such as phenolics, sterols and proteins have been also isolated from these plants (Bozorgi et al., 2017). Flavonoides are the main phenolic compounds identified in Drimia species and flavonoids such as Cyanidin 3-monoglucoside and pelargonidin 3-monoglcoside with caffeic and ρ-cumaric acid were isolated from drimia species (Bozorgi et al., 2017). According to chemical compounds of drimia, total phenolic and flavonoid contents of *D. maritima* was determined in this study. The chemical compounds of squill bulb such as cardiac glycosides or flavonoids may be responsible for anticonvulsant activity of this plant. There are some flavonoids such as apigenin, rutin and vitexin with anticonvulsant activity (Zhu et al., 2014). Future studies on squill bulb can determine the active anticonvulsant constituents of this plant.

PTZ-induced seizure can reduce γ-aminobutyric acid (GABA) level in the cortex. GABA has been reported as the dominant inhibitory neurotransmitter in the central nervous system of mammals and has been involved in convulsions by increasing the chloride-ion conductance via the opening of the chloride-ion channel and so, it mediates the inhibition of neuronal responsiveness and activity (Corda et al., 1990; Riazi et al., 2004). The findings of the present study reveals that D. *maritima* oxymel might have delayed the occurrence of PTZ-induced seizure and reduced its duration probably by acting on GABAergic system. Clarification of the mechanism of its antiseizure activity needs further study.

Conclusion

This study provides scientific evidence for using *D. maritima* oxymel in treating seizure of epilepsy in Iranian traditional medicine. Therefore, further studies may utilize this plant as an anticonvulsant supplement for treating epilepsy.

Conflicts of interest

There is no conflict of interest to declare.

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