BJPS

Brazilian Journal of
Pharmaceutical Sciences

http://dx.doi.org/10.1590/s2175-97902022e20066

Article

The antiepileptic activity of Safranal in kindling model of epilepsy in male rats

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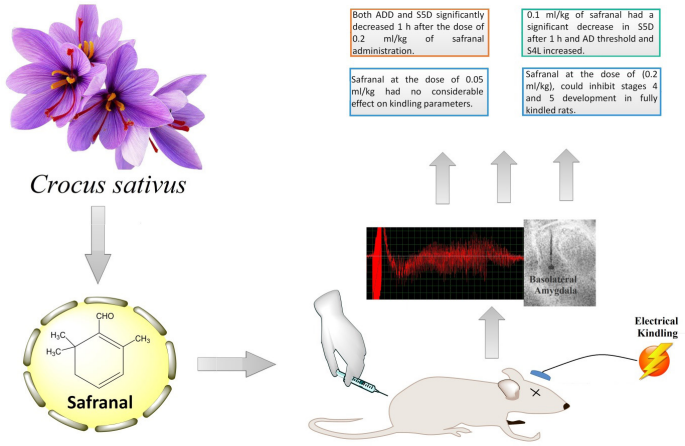
Recent studies suggested that safranal exerts anticonvulsant properties. The present study aimed to investigate the effect of safranal on epileptic activities in the amygdala electrical kindling model in male rats. Animals were implanted with a recording electrode on the skull and a tripolar in the amygdala. After 10 days of recovery, the afterdischarge (AD) threshold of each animal was determined and stimulated once daily the AD threshold for full kindling development. Then, parameters including afterdischarge duration (ADD), stage 4 latency (S4L), stage 5 duration (S5D), and stimulation threshold were determined before and after injection of safranal (0.05, 0.1, 0.2 ml/kg; i.p). While the dose of 0.05 ml/kg had no significant effect, the dose of 0.1 ml/kg increased the AD threshold as well as S4L and decreased the S5D (P<0.05). Injection of 0.2 ml/kg of the safranal significantly decreased the ADD and S5D (P<0.05) and 83.3% of animals had no stage 4 and stage 5 of kindling (P<0.001). Based on the obtained data safranal has anticonvulsant effects dose-dependently. It seems that a dose of 0.2 ml/kg is the minimum effective dose. Further investigation is warranted to conduct the clinical implications for the treatment of epileptic disorders.

Keywords: Safranal. Epilepsy. Electrical Kindling. Amygdala. Seizure. Male Rat.

Key Messages: 0.1 ml/kg of safranal had a significant decrease in S5D after 1 h and the AD threshold and S4L increased.
Both ADD and S5D significantly decreased 1 h after the dose of 0.2 ml/kg of safranal administration. Safranal at the dose of (0.2 ml/kg), could inhibit stages 4 and 5 development in fully kindled rats.

Graphical abstract



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INTRODUCTION

Epilepsy is one of the ancient neurological disorders, with high prevalence, known to human beings still with unclear concepts for its origin and pathophysiology (Stafstrom, Carmant, 2015). It seems sudden excessive disorganized discharges from the cerebral neurons occur frequently (French, Pedley, 2008). The main cause is excessive extracellular Ca^{2+} which leads to uncontrolled voltage-gated Na^{+} channel opening, high intracellular Na^{+} concentration, and consequent recurrent action potentials (Escayg, Goldin, 2010; Armijo *et al*, 2005).

In nearly 70% of epileptic patients, seizures are controlled through consuming different synthetic medications (Jacob, Nair, 2016). These drugs act via different mechanisms such as affecting membrane ion channels, gamma-aminobutyric acid (GABA), or glutamate transmission (O'Dell *et al*, 2012). A number of common anticonvulsant drugs seem to prolong inactive Na^{+} channels phase and so refractory period. (O'Dell *et al*., 2012). Unfortunately, in many cases, these conventional anticonvulsant agents are not able to control seizures efficiently (Kwan, Schachter, Brodie, 2011). Besides the risk of neurotoxicity or other adverse side effects of anti-epileptic drugs may influence their long term administration (Ijff *et al*., 2015), the study and development of new safer neuroprotective compounds with anticonvulsant activities are indispensable (St Louis, 2009). Traditional medicine and medicinal plants have been considered in this area (Zagaja *et al*., 2016). Animal studies have confirmed the antiepileptic activity of some herbal extracts considering their beneficial usage in the management of seizures (Bhosle, 2013; Nassiri-Asl, Shariati-Rad, Zamansoltani, 2007).

Crocus sativus and its extract saffron) of which crocin, and safranal are the main components) have been largely used in traditional medicine for its various beneficial properties such as anti-diabetic, pain-suppressing, antioxidant, neuroprotective and anti-apoptotic effects (Zeka *et al*., 2015; Hosseinzadeh, Younesi, 2002; Hosseinzadeh, Sadeghnia, 2007; Amin, Hosseinzadeh, 2012; Bie *et al*., 2011). Recently, Safranal, the responsible constituent for the characteristic saffron odor has claimed to have anticonvulsant effects in both maximal electroshock (MES) and pentylenetetrazole (PTZ) animal models. Unlike crocin, safranal has shown to be able to control both tonic and colonic phases of PTZ-induced seizures (Hosseinzadeh, Sadeghnia, 2007; Hosseinzadeh, Talebzadeh, 2005). Other neuroprotective effects of safranal have been revealed by its ability to exert antioxidant activity and attenuating cerebral ischemia in rat hippocampus (Khazdair *et al.,* 2015). Despite the previous study on the antiepileptic effect of saffron, the exact effect of this herbal potential intervention has not been identified well.

To study the neurobiology of seizure and investigating new and effective treatments, animal models of kindling are more appropriate. Repeated applications of short-term, recurrent, and low-intensity electrical stimulation lead to the gradual development of electrographic and behavioral seizure. This kind of generalized seizure is very similar to human complex seizures with precise quantitative parameters. There was no available study on the exact effect of safranal on the threshold and different stages of tonic -colonic seizure and this is the first study designed to investigate the effect of safranal administration in the kindling model of epilepsy in male rats.

MATERIAL AND METHODS

Animals

Forty-five adult Wistar male rats (280-320 g body weight) were obtained from the Pasteur Institute of Iran. The animals were kept in a room with a constant temperature of 23±1°C on 12:12 light: dark schedule with free access to food and water. They were kept according to the guidelines for the care and use of laboratory animals (Jones-Bolin, 2012). All experiments were carried out in accordance with the ethical principles of laboratory animal care (NIH publication) and laws of animal protection (National Research Council, 2010). Also, at the end of procedures, all animals were euthanized by diethyl ether anesthesia.

Electrode implanting surgery

Animals were anesthetized by ketamine (50 mg/kg, i.p.) and lidocaine 2% (10 mg/kg, i.p) and implanted with insulated a bipolar inspiring and monopolar recording

electrodes (twisted into a tripolar configuration) in the basolateral amygdala (coordinates: A, 2.5 mm; L, 4.8 mm and 7.5 mm below dura) of the right hemisphere (Saberi *et al*., 2020). The electrodes (stainless steel, Teflon-coated, 127 μm in diameter, AM-Systems, USA) were protected except at the tip. Two other electrodes were attached to skull screws, positioned above the left cortical surface as a reference and differential electrodes. The electrical stimulation procedure was started 10 days after the implantation of electrodes (Saberi, Pourgholami, Jorjani, 2001).

Kindling procedure

After discharge (AD) threshold was determined in basolateral amygdala by a 2 sec, 60 Hz monophasic square wave stimulus of 1 msec per wave (by Electromodule D3111, ScienceBeam Institute, Tehran, Iran). During the first stimulation session, the minimum stimulation threshold (AD threshold) was determined by an ascending series of 25 μA incremental stimulation (maximum to 400 μA) and 5-minute intervals until at least 5 sec AD recording was achieved as previously described (Saberi, Rezvanizadeh, Bakhtiarian, 2008). Evoked responses were amplified, filtered, and digitized (at 10 kHz) using a PC-based data acquisition system and recording software (Electromodule D3111 and NeuroTrace provided by Science Beam Institute, Tehran, Iran) and were continuously monitored and stored.

Antiepileptic activity measurement

The animals were stimulated once daily at the AD threshold intensity until five sequential stages 5 of seizures occurred. Kindling parameters including, AD duration (ADD), the latency to the onset of bilateral forelimb clonus (S4L), and stage 5 duration (S5D) were recorded daily, during the kindling process. Seizure duration was measured from the beginning until the end of seizure behavior. ADD was measured after the stimulation (2 sec) until the end of ADD. The duration of epileptiform activities, after discharges and the behavioral progression of kindling (stages 1-5 according to the Racine's scale) were monitored. Briefly, stages included, stage 1 mouth and facial movement; stage 2, head nodding; stage 3, forearm or limbs clonus; stage 4, rearing; and stage 5, rearing and falling and loss of postural control (Wu *et al*., 2019).

Experimental design

On the first day, the preliminary record from each animal was obtained as control. To test the selected solvent and its probable effects on kindling parameters, on the second day, sesame oil (1 ml/kg, i.p.) was injected and subsequent electrical recordings were obtained. Then on the following day, animals received the treatment and one hour later electrical stimulation performed, and all kindling parameters (cited above) were recorded.

The animals were classified into four groups (N=5). Fully kindled rats were subjected either to sesame oil (1 mg/kg) or different safranal doses (0.05, 0.1, and 0.2 ml/kg in groups 2-4, respectively) intraperitoneally on the following day.

Safranal administration

Safranal (Sigma, 132.193 mg/ml) was dissolved in sesame oil and injected at the doses of either 0.05, 0.1, or 0.2 ml/kg intraperitoneally 60 min before kindling stimulation.

Statistical analysis

All obtained data are expressed as the mean ± S.E.M.. The difference between every two non-dependent groups was analyzed using paired Student's t-test by SPSS software (version 19.0). A repeated-measures ANOVA was used to determine changes in cumulative ADD (the sum of after discharge durations recorded). A P value of less than 0.05 was considered a significant difference.

RESULTS

After ten days as recovery from the surgery, the animals were gone under the kindling process to reach full kindling as cited above. All animals were considered fully

kindled with five consecutive stage 5 seizure. Although lower doses of the safranal had no behavioral effects, five minutes after the dose of 0.2 ml/kg, hyperactivity and agitation was observed and followed by a sedation period. The animals become plethoric at the nose, ears, and extremities after 15 minutes.

Effects of solvent on kindling parameters

There was no significant change in parameters (AD threshold, ADD, S4L, and S5D) when these factors were evaluated before and after solvent (sesame oil) administration as well as the control group (Figure 1).

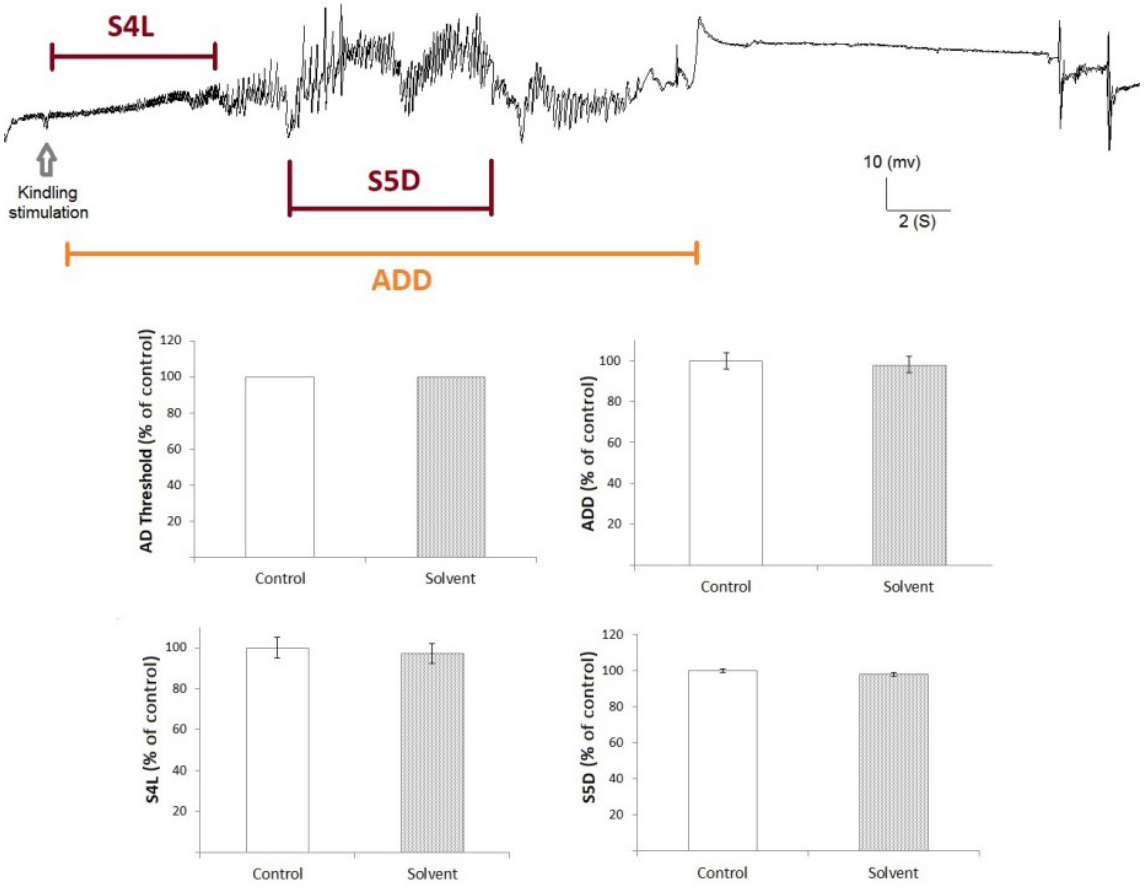

FIGURE 1 - A Sample records of discharges and the effects of solvent on kindling parameters (AD threshold, ADD, S4L, and S5D). These factors were evaluated before and after the administration of sesame oil.

Effects of safranal on kindling parameters

Figure 2 demonstrates the effects of different doses of safranal on kindling parameters. Safranal at the dose of 0.05 ml/kg had no considerable effect on kindling parameters including AD threshold, ADD, and S4L when compared to the control group one h after administration.

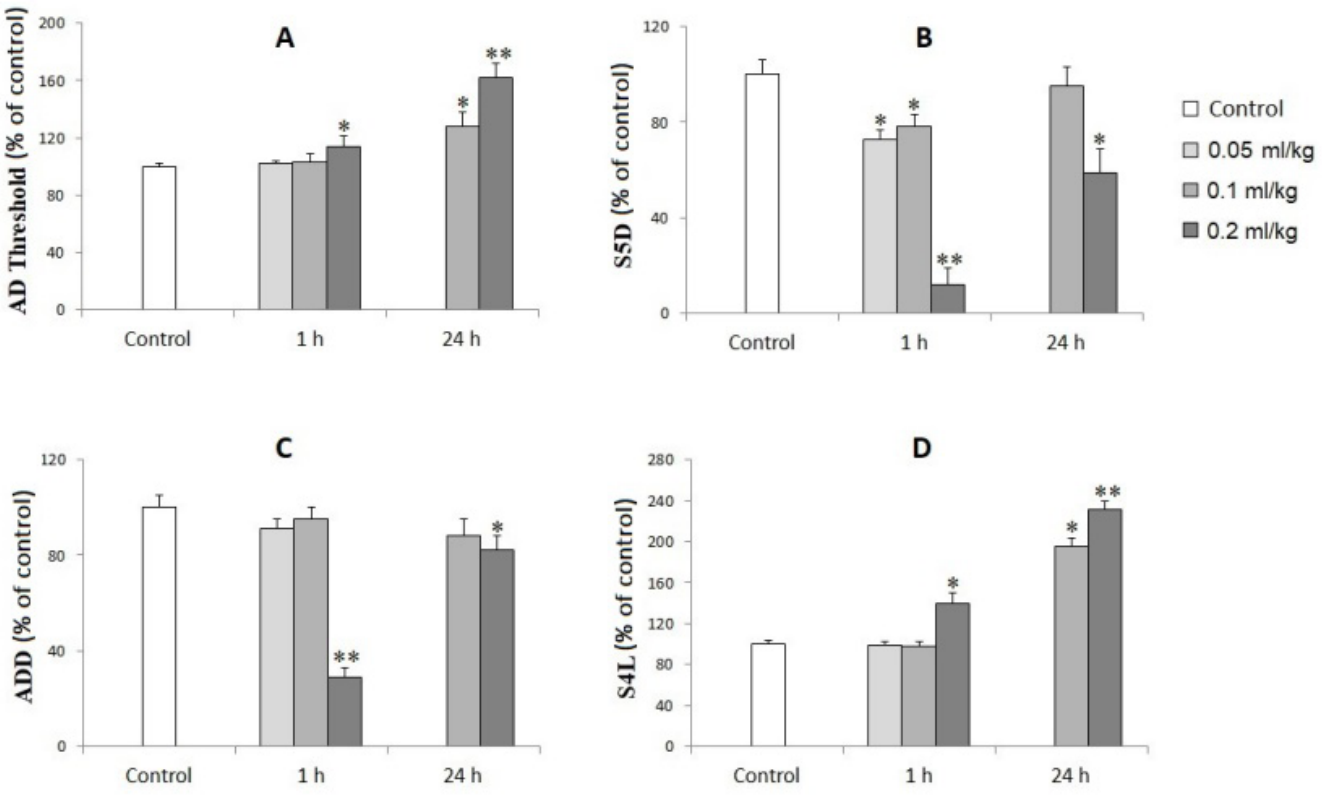

FIGURE 2 - Effect of intraperitoneal administration of safranal (0.05, 0.1 and 0.2 ml/kg) after 1 and 24 h on AD threshold (A), S5D (B), ADD (C) and S4L (D) in the electrical amygdala kindling model of epilepsy in intact male rats. Each group of animals received daily either safranal or vehicle (control). Data are expressed as a percent of control (mean ± S.E.M.). * Indicates significant in comparison to the control group, $P<0.05$ and **, $P<0.001$ (N=5).

Effects of safranal (0.1 ml/kg) on kindling parameters

The AD threshold, S5D, and ADD were evaluated 1 hour and 24 hours after the dose of 0.1 ml/kg of safranal (i.p.) administration. While, the AD threshold and S4L increased significantly after 24 hours ($P<0.05$), there was a significant decrease in S5D after 1 h and non-significant decrease in both ADD and S5D after 24 h (Figure 2).

Effects of safranal (0.2 ml/kg) on kindling parameters

Both ADD and S5D significantly decreased 1 h after the dose of 0.2 ml/kg of safranal administration ($P<0.001$). These effects remained significant for 24 h ($P<0.05$) when compared to the control group (Figure 1). Inhibition of 84% was observed 1 h after the dose of 0.2 ml/kg of safranal administration on S5D and stages 4 and 5 (as generalized seizures) were not developed. Conversely, the AD threshold and S4L increased from 1 h and remained significant until 24 h ($P<0.05$).

The effect of safranal administration (different doses) on kindling stage

Safranal at the dose of (0.2 ml/kg), could inhibit stages 4 and 5 development in fully kindled rats ($P<0.001$) when compared to the control group (Figure 3).

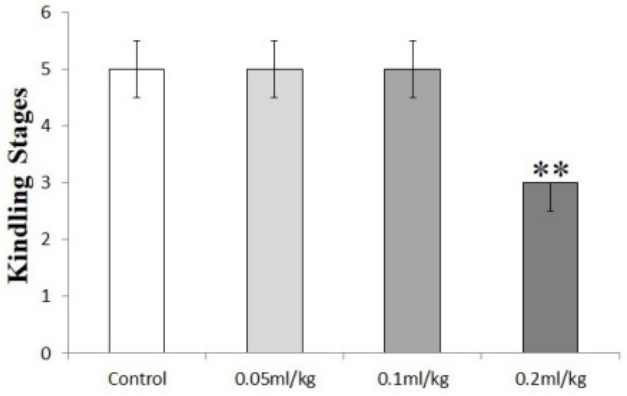

FIGURE 3 - Effects of different intraperitoneal doses of safranal (0.05, 0.1, and 0.2 ml/kg) on different stages of the electrical amygdala kindling model of epilepsy in intact male rats. Kindling stages were evaluated from zero to stage 5 based on the Racine's Scale as cited above. ** Indicates significant, ($P<0.01$) in comparison to the control group.

DISCUSSION

Epilepsy is an important widespread neurological disorder with unknown precise origin which is characterized by recurrent seizure attacks (Perez, LaFrance, 2016). Anticonvulsant agents prevent seizure recurrence and alleviate clinical and electrical seizure activity (Ventola, 2014; Brodie, Sills, 2011). Because of various unpreventable side effects that have been reported for anticonvulsant drugs, it seems it is necessary to research on new natural neuroprotective compounds with fewer adverse reactions (Schmidt, Beyenburg, 2009).

Based on the result of previous investigations, in this study safranal, was chosen as a possible antiepileptic agent (Hosseinzadeh, Sadeghnia, 2007). The literature review shows that safranal as an active volatile oil component of saffron, has promising and attractive effects on neuropsychological disorders (Rezaee, Hosseinzadeh, 2013). In the present study, the effects of safranal on different known kindling parameters in kindled male rats were evaluated.

Full kindling acquisition after proper stimulation occurred and animals experienced complete end-stage of seizure. Our findings revealed that safranal administration exerts anticonvulsive properties in this model dose-dependently. Also, safranal at the dose of 0.1 ml/kg could raise the AD threshold successfully for 24 hours as the AD threshold increased over 35% after safranal administration. This means that more powerful electrical stimulations than the threshold were applied to induce at least 5 sec ADD in safranal treated animals. The S4L prolongation in safranal treated animals indicates that generalized seizures may develop at least with more delay. Moreover, the prevention of stages 4 and 5 is exact and strong evidence of anti-convulsive action of safranal against generalized seizures. So safranal probably prevents the progression of the complex partial seizures to the generalized seizures as well.

Moreover, there was a significant protective effect of safranal on ADD dose-dependently. These observed properties display the potential inhibitory and protective effects of safranal against complex partial seizure as well as other attacks. Complete systemic seizure (equivalent to 4^{th} stage of kindling), was delayed about 2 times in comparison to control which can be along with its ability to inhibit generalized seizure. This effect which was obvious until 24 hours following safranal injection, indicates a long protective action of the safranal treatment. The higher dose of safranal (0.2 ml/kg) showed anti-seizure and protective effects in this animal model of epilepsy as could change all kindling parameters toward the antiepileptic activities of this material. Safranal diminished electrical discharge of neurons about 75%, which weakens the probable conduction of inspired signals to other regions of the brain. As a result, stages 4 and 5 of kindling did not happen and suppressed for about 90%.

These results were compatible with previous studies on saffron as its active components exhibited their anticonvulsant effects in PTZ and MES seizure models in rats (Rezaee, Hosseinzadeh, 2013; Srivastava *et al*., 2010). Intra-peritoneal administration of Safranal could inhibit chemical kindling induced by PTZ, in a dose-dependent manner while there was no protective effect when safranal was administered intracerebroventricularly. Pentylenetetrazol induced seizure was attenuated by morphine, diazepam, and safranal, contrasted with flumazenil and naloxone administration. This fact suggested $GABA_A$ involvement in the safranal protective effect (Rezaee, Hosseinzadeh, 2013; Hosseinzadeh, Sadeghnia, 2007). It had been shown that safranal can

exert anti-seizure effect dose-dependently when absence seizure induced by gamma-butyrolactone, baclofen, PTZ, and picrotoxin (Sadeghnia *et al*., 2008). On the other hand, safranal has reduced flunitrazepam bounding in cortex, hypothalamus, and hippocampus which again indicates the probable role of the $GABA_A$ system (Nassiri-Asl, Shariati-Rad, Zamansoltani, 2007).

In all mentioned investigations chemical kindling with qualitative results has been reported. There are many advantages when electrical kindling is applied. Its pattern is very similar to human complex partial seizure and animals are kindled irreversibly with almost constant parameters. Quantitative data can be achieved and it is possible to observe and record animal behavior when stimulated simultaneously. By electrical kindling, the exact cerebral threshold is estimated as a valuable seizure factor (Kandratavicius *et al*., 2014). All these advantages have been achieved along with our study and valuable data regard to safranal protective effect against convulsion attained. Also, more investigation to reveal the precise mechanism and probable ability of safranal on the prevention of kindling acquisition is necessary.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES

Amin B, Hosseinzadeh H. Evaluation of aqueous and ethanolic extracts of saffron, *Crocus sativus* L., and its constituents, safranal and crocin in allodynia and hyperalgesia induced by chronic constriction injury model of neuropathic pain in rats. Fitoterapia. 2012;83(5):888-95.

Armijo JA, Shushtarian M, Valdizan EM, Cuadrado A, Adin J. Ion channels and epilepsy. Curr Pharm Des. 2005;11(15):1975-2003.

Bhosle V. Anticonvulsant and antioxidant activity of aqueous leaves extract of *Desmodium triflorum* in mice against pentylenetetrazole and maximal electroshock induced convulsion. Rev Bras Farmacogn. 2013;23(4):692-98.

Bie X, Chen Y, Zheng X, Dai H. The role of crocetin in protection following cerebral contusion and in the enhancement of angiogenesis in rats. Fitoterapia. 2011;82(7):997-1002.

Brodie MJ, Sills GJ. Combining antiepileptic drugs—Rational polytherapy? Seizure. 2011;20(5):369-75. doi: https://doi.org/10.1016/j.seizure.2011.01.004.

Escayg A, Goldin AL. Sodium channel SCN1A and epilepsy: mutations and mechanisms. Epilepsia. 2010;51(9):1650-8. doi: 10.1111/j.1528-1167.2010.02640.x.

French JA, Pedley TA. Initial Management of Epilepsy. N Engl J Med. 2008;359(2):166-76. doi: 10.1056/NEJMcp0801738.

Hosseinzadeh H, Sadeghnia HR. Protective effect of safranal on pentylenetetrazol-induced seizures in the rat: Involvement of GABAergic and opioids systems. Phytomedicine. 2007;14(4):256-62. doi: https://doi.org/10.1016/j.phymed.2006.03.007.

Hosseinzadeh H, Talebzadeh F. Anticonvulsant evaluation of safranal and crocin from *Crocus sativus* in mice. Fitoterapia. 2005;76(7-8):722-4. doi: https://doi.org/10.1016/j.fitote.2005.07.008.

Hosseinzadeh H, Younesi HM. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. BMC Pharmacol. 2002;2:7.

IJff DM, Kinderen R, Vader C, Majoie MH, Aldenkamp AP. Subjectively perceived side-effects of anti-epileptic drugs in chronic refractory epilepsy. Adv Pharmacoepidemiol Drug Saf. 2015;4(4):186.

Jacob S, Nair AB. An updated overview on therapeutic drug monitoring of recent antiepileptic drugs. Drugs R D. 2016;16(4):303-16.

Jones-Bolin S. Guidelines for the care and use of laboratory animals in biomedical research. Curr Protoc Pharmacol. 2012;59(1):A-4B.

Kandratavicius L, Balista PA, Lopes-Aguiar C, Ruggiero RN, Umeoka EH, Garcia-Cairasco N, et al. Animal models of epilepsy: use and limitations. Neuropsychiatr Dis Treat. 2014;10:1693-705. doi: 10.2147/NDT.S50371.

Khazdair MR, Boskabady MH, Hosseini M, Rezaee R, M. Tsatsakis A. The effects of *Crocus sativus* (saffron) and its constituents on nervous system: A review. Avicenna J Phytomed. 2015;5(5):376-91.

Kwan P, Schachter SC, Brodie MJ. Drug-resistant epilepsy. N Engl J Med. 2011;365(10):919-26.

Nassiri-Asl M, Shariati-Rad S, Zamansoltani F. Anticonvulsant effects of aerial parts of *Passiflora incarnata* extract in mice: involvement of benzodiazepine and opioid receptors. BMC Complem Altern M. 2007;7:26. doi: 10.1186/1472-1468.

National Research Council (United States). Guide for the care and use of laboratory animals. National Academies Press. 2010, 27.

O'Dell CM, Das A, Wallace Gt, Ray SK, Banik NL. Understanding the basic mechanisms underlying seizures in mesial temporal lobe epilepsy and possible therapeutic targets: a review. J Neurosci Res. 2012;90(5):913-24. doi: 10.1002/jnr.22829.

Perez DL, LaFrance WC. Nonepileptic seizures: an updated review. CNS Spectr. 2016;21(3):239-46.

Rezaee R, Hosseinzadeh H. Safranal: From an Aromatic Natural Product to a Rewarding Pharmacological Agent. Iran J Basic Med Sci. 2013;16(1):12-26.

Saberi F, Bahrami F, Saberi M, Mashhadi Akbar Boojar M. The pro-convulsant effects of diazinon low dose in male rats under amygdala kindling. Drug Chem Toxicol. 2020;4:1-8.

Saberi M, Pourgholami MH, Jorjani M. The acute effects of estradiol benzoate on amygdala-kindled seizures in male rats. Brain Res. 2001;891(1-2):1-6.

Saberi M, Rezvanizadeh A, Bakhtiarian A. The antiepileptic activity of *Vitex agnus castus* extract on amygdala kindled seizures in male rats. Neurosci Lett. 2008;441(2):193-6. doi: 10.1016/j.neulet.2008.06.034.

Sadeghnia HR, Cortez MA, Liu D, Hosseinzadeh H, Snead OC, 3rd. Antiabsence effects of safranal in acute experimental seizure models: EEG and autoradiography. J Pharm Pharm Sci. 2008;11(3):1-14.

Schmidt D, Beyenburg S. Chapter 7 - Antiepileptic drugs. In: Aronson JK, editor. Side Effects of Drugs Annual: Elsevier; 2009. p. 105-48.

Srivastava R, Ahmed H, Dixit RK, Dharamveer, Saraf SA. *Crocus sativus* L.: A comprehensive review. Pharmacogn Rev. 2010;4(8):200-8. doi: 10.4103/0973-7847.70919.

St Louis EK. Minimizing AED adverse effects: Improving quality of life in the interictal state in epilepsy care. Curr Neuropharmacol. 2009;7(2):106-14. doi: 10.2174/157015909788848857.

Stafstrom CE, Carmant L. Seizures and epilepsy: an overview for neuroscientists. Cold Spring Harb Perspect Biol. 2015;5(6):a022426. doi: 10.1101/cshperspect.a022426.

Ventola CL. Epilepsy Management: Newer Agents, Unmet Needs, and Future Treatment Strategies. P T. 2014;39(11):776-92.

Wu YV, Liu J, Chen Z, Burnham WM. Benzyl alcohol suppresses seizures in two different animal models. Neurol Res. 2019;41(7):652-657.

Zagaja M, Andres-Mach M, Patrzylas P, Pyrka D, Szpringer M, Florek-Łuszczki M, et al. Influence of xanthotoxin (8-methoxypsoralen) on the anticonvulsant activity of various novel antiepileptic drugs against maximal electroshock-induced seizures in mice. Fitoterapia. 2016;115:86-91. doi: 10.1016/j.fitote.2016.09.020.

Zeka K, Ruparelia KC, Continenza MA, Stagos D, Vegliò F, Arroo RR. Petals of *Crocus sativus* L. as a potential source of the antioxidants crocin and kaempferol. Fitoterapia. 2015;107:128-34. doi: 10.1016/j.fitote.2015.05.014.

Received for publication on 06th March 2020
Accepted for publication on 08th December 2020