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Original Article

Pre-gestational feeding of thymoquinone suppressed pentylenetetrazole-induced generalized seizure while potentiated focal seizure in rat offspring

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Abstract

Introduction: Pre-gestational nutrition, before and during pregnancy, might play a key role in nervous system. This study aimed to investigate effect of pre-gestational administration of thymoquinone (TQ) on pentylenetetrazole (PTZ)-induced seizure in rat offspring.

Methods: Thirty-two female Wistar rats were divided to four groups (n=8) as follows: 1-control (received one ml ethanol 25% by gavage for seven constitutive days), 2-TQ10, 3-TQ 40 and 4-TQ 80. The rats in the groups 2, 3 and 4 received 10, 40 and 80mg/kg TQ dissolved in one ml ethanol 25% by gavage for seven constitutive days, respectively. The day after finishing the TQ administration, each female rat was mated with a sexually experienced male rat. The pregnant rats were housed in groups of four per cage until the day 20 of gestation. Then, the rats were individually transferred to a separate cage and the same conditions were applied for all of them. After parturition, the pups were counted, weighed and culled to eight in each litter. On postnatal day 14 (P14) and P21, the pups were subjected to PTZ-induced seizure.

Results: Latency of the first seizure decreased in the TQ40 and TQ80 groups and the duration of focal seizure increased both at P14 and P21. The progress of seizure stages and duration of tonic-clonic seizures were suppressed in TQ-treated rats.

Conclusion: Exposure to TQ before conception potentiates focal seizure while suppresses generalized PTZ-induced seizure in rat offspring. TQ significantly changed the PTZ-induced seizure pattern in favor of focal seizure.

Keywords:
Thymoquinone;
Seizure;
Pentylenetetrazole;
Pre-gestation

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Introduction

Women's nutrition, before and during pregnancy, might play a key role in their nervous system and reproductive health and is recognized to have significant impacts on optimizing pregnancy outcomes (Ebisch et al., 2006; Vilela et al., 2014;

Schoenaker et al., 2015). Several human and animal studies have suggested that pre-gestational nutrition can influence the hypothalamic-pituitary-adrenal axis which consequently influences the outcomes such as pre-eclampsia and preterm delivery (Bloomfield et al., 2004). Furthermore, healthy diet during pre-gestational period is negatively correlated with depression symptoms, so that healthy diet reduces the depression symptoms (Vilela et al., 2014). Herbal products have been a main source of remedies and they are extensively used worldwide. Because of their natural origin, they have been considered harmless and without any side effects. Although their side effects are less recurrent than those of synthetic drugs, the notion that natural products are fully safe and without any adverse effects is incorrect (Salarinia et al., 2016). The potential effect and/or toxicity of drugs in pre-conception and pregnancy should be tested before their use in pregnant mothers and/or in women who plan to get pregnant in near future. In the traditional treatment of neurological diseases specially seizure and epilepsy, many herbal substances such as *Coriandrum sativum* (Hosseinzadeh and Khosravan, 2002), *Crocus sativus* (Hosseinzadeh and Madanifard, 2000), *Matricaria recutita* (Heidari et al., 2009) and *Lavandula officinalis* (Rahmati et al., 2013) as well as *Nigella sativa* (NS) have been studied (Ilhan et al., 2005; Mostafa, 2013). Therefore, the possible adverce effects of all natural products need to be tested before their clinical applications. NS, commonly known as black cumin, belongs to the botanical family of Ranunculaceae, which is widely cultivated in the Mediterranean region. Pharmacological studies have demonstrated that NS and thymoquinone (TQ) exhibit a broad range of biological effects, including neuroprotective, cardioprotective, nephroprotective, hepatoprotective, anti-cancer, anti-inflammatory and anti-microbial actions. Based on its anti-microbial effect, a vaginal suppository form of NS is in clinical use for vaginal fungal infection (Salarinia et al., 2016). Also, there are many reports documenting that NS oil has an anti-convulsant effect (Akhondian et al., 2007; Raza et al., 2008). The main component of this oil, TQ, has many analogues including parabenzooquinone, 2-methyl parabenzoquinone and 2-isopropyl parabenzoquinone. These compounds have also been studied in terms of anticonvulsant effects (Hosseinzadeh et al., 2005; Sousa et al., 2011; Abdel-Rahman et al., 2013). Thus, based on a broad range of biological effects of NS oil, its use is possible in women before and during pregnancy. Hence, its possible adverse effects need to be tested before its clinical applications in animal studies. Though several studies have examined the anticonvulsant effects of TQ, there is still no evidence available indicating the effects of mother fed with NS active component (TQ) during pre-gestational period on their offspring, particularly on susceptibility of children to pentylenetrazole (PTZ)-induced seizure. Therefore, the present study designed to investigate the effect of exposure to TQ before conception on PTZ-induced seizure in rat offspring.

Materials and methods

TQ (CAS no. 490-91-5, Cayman, USA), PTZ (Sigma Aldrich, Germany) and ethanol (Merck, Germany) were used in this experimental study.

Study design

Thirty-two virgin female Wistar rats 180-200g (10-week-old) were purchased from animal facility at Urmia University of Medical Sciences, Urmia, Iran. All procedures for animals were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised in 1985) and were approved by the Ethical Committee of Urmia University of Medical Sciences, Iran. The rats were housed in groups of four per cage and kept in standard conditions as follows: standard 12 h light/dark cycle, 22±2°C and food and water *ad libitum*. One week after maintenance and compatibility with the laboratory, the rats were divided into four groups (n=8 per group) as follow: 1-control (received one ml ethanol 25% by gavage for 7 constitutive days), 2-TQ10, 3-TQ40 and 4-TQ80. The rats in the groups 2, 3 and 4 received 10, 40 and 80mg/kg TQ dissolved in one ml ethanol 25% by gavage for seven constitutive days, respectively. TQ was administered for 7 days to cover a full estrous cycle of rats which is about 5 days (Mahmoodkhani et al., 2018). The day after finishing the TQ administration, each female rat was mated with a sexually experienced male rat in a separate cage. The female rats were checked for vaginal plaque and positive history of vaginal plaque was considered as

the first day of pregnancy. The pregnant female rats were housed in groups of four per cage and kept in standard conditions as stated earlier in this section until the day 20 of gestation. Then the rats were individually transferred to a separate cage and the same conditions were applied for all of them. After parturition, the pups were counted, weighed and culled to eight in each litter to minimize the effect of unequal litter size on pups' nursing, development and growth. The day of delivery was considered as the postnatal day 1 (P1). On P14 and P21, the pups were subjected to PTZ-induced seizure. Both male and female pups were selected (16 rats per group, one male and one female pup per dam).

PTZ-induced seizure

PTZ is frequently used as a validated seizure model in rodents and previously explained by others (Kandratavicius et al., 2014). Briefly, the rats received PTZ (45mg/kg, IP) in the morning at 8:00 to 11:00 either on P14 or P21 and were transferred to a glass chamber (30×30×30cm) for their behaviors to be measured. Convulsive behaviors of the rats were monitored for 60min and seizure score was determined for each rat in accordance with the five-stage criterion developed by (Mahmoodkani et al., 2018). 0=normal; 1=immobilization, sniffing; 2=head nodding, facial and forelimb clonus (short myoclonic jerk); 3=continuous myoclonic jerk and tail rigidity; 4=generalized limbic seizures with kangaroo posture or violent convulsion and 5=continuous generalized seizures (tonic or tonic-clonic convulsions). In addition, the latency to the first seizure, first tonic-clonic seizure, duration of tonic-clonic seizures and the latency to the first leg extension were also observed and recorded.

Data analysis

Data were imported to the SPSS software Version 22 and the data distribution was checked using Kolmogorov–Smirnov test. Normally distributed data were analyzed using parametric techniques. One-way analysis of variance (ANOVA) was used for multiple comparisons of body weight followed by Tukey's post hoc test when required. Two-way ANOVA was used for analysis of data related to seizure stages followed by Bonferroni post hoc test when required. The data, which were not normally distributed (latency, duration and number of tonic-clonic seizures) were analyzed using nonparametric tests such as Kruskal–Wallis and/or Mann-Whitney U tests. The results were expressed as mean±SEM and $P<0.05$ was considered significant.

Results

At first, the data from both sexes were separately analyzed in terms of body weight and seizure parameters. There was no significant difference between male and female pups; therefore, the data

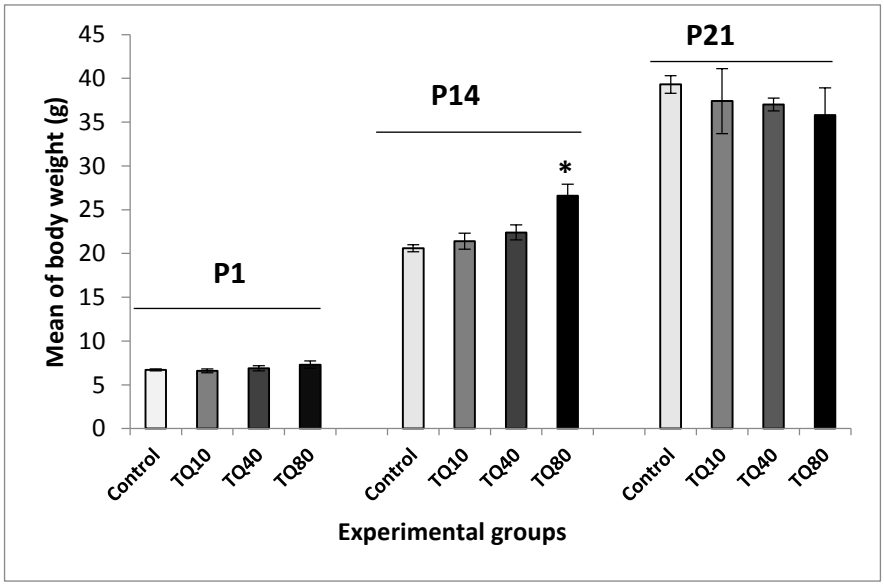

Fig.1. Rat pup's body weight at one, 14 and 21 days after birth (P1, P14 and P21, respectively). The values show mean±SEM, control (pre-gestational control), TQ10 (thymoquinone 10mg/kg), TQ40 (thymoquinone 40mg/kg) and TQ80 (thymoquinone 80mg/kg). *shows significance with control at the same day ($P<0.01$).

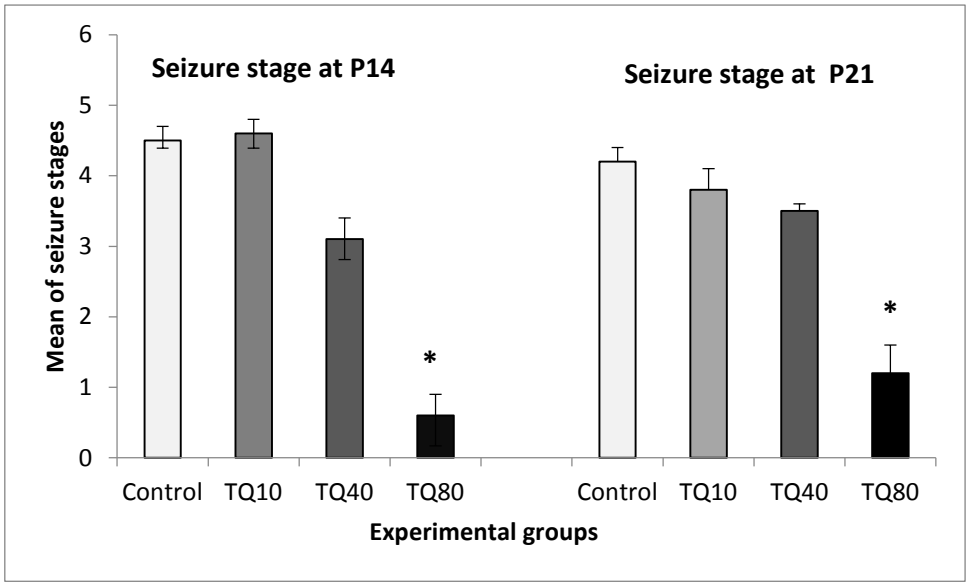

Fig.2. Sizure stage at 14 and 21days after birth (P14 and P21). The values show mean±SEM, control (pre-gestational control), TQ10 (thymoquinone 10mg/kg), TQ40 (thymoquinone 40mg/kg) and TQ80 (thymoquinone 80mg/kg); *shows significance with all groups at the same age (P<0.001, two-way ANOVA and Bonferroni). Effect of age was not significant.

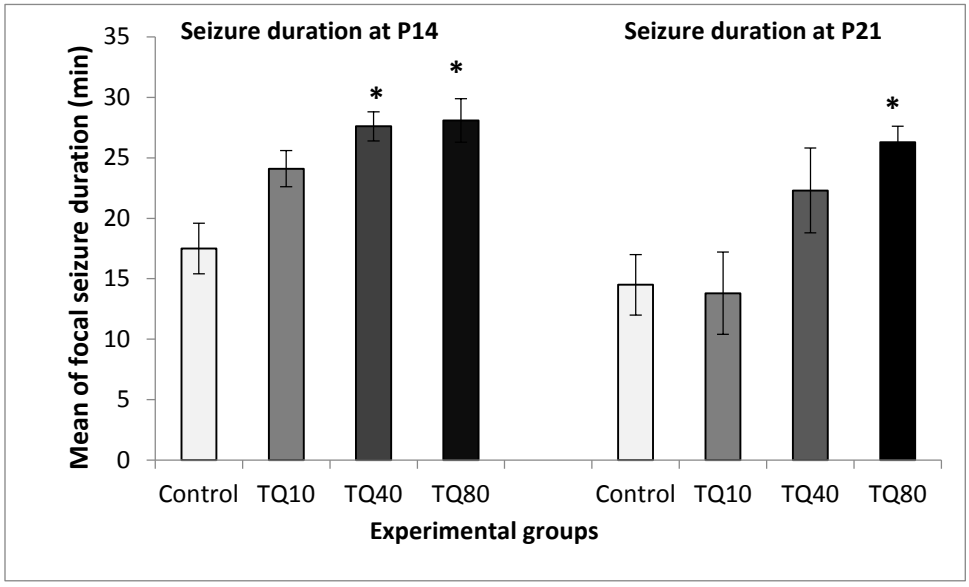

Fig.3. Sizure duration at 14 and 21 days after birth (P14 and P21). The values show mean±SEM, TQ10 (thymoquinone 10mg/kg), TQ40 (thymoquinone 40mg/kg) and TQ80 (thymoquinone 80 mg/kg); *Show the significance with control at the same day (Kruskal–Wallis, P<0.05).

from the both sexes were combined and analyzed together.

Effect of pre-gestational administration of TQ on body weight in rat offspring

At P1, there was a non-significant increase of pup's body weight in TQ80 group compared to the control group. At P14, the mean body weight significantly increased in TQ80 group compared to the control rats (P<0.01). There was no significant difference in pup's body weight at P21. The overall result showed that pre-gestational administration of TQ 80mg/kg increased the pup's body weight until P14.

Effect of pre-gestational administration of TQ on seizure stages in rat offspring

Data related to seizure stages were analyzed by two-way ANOVA for two factors of TQ and age. Effect of

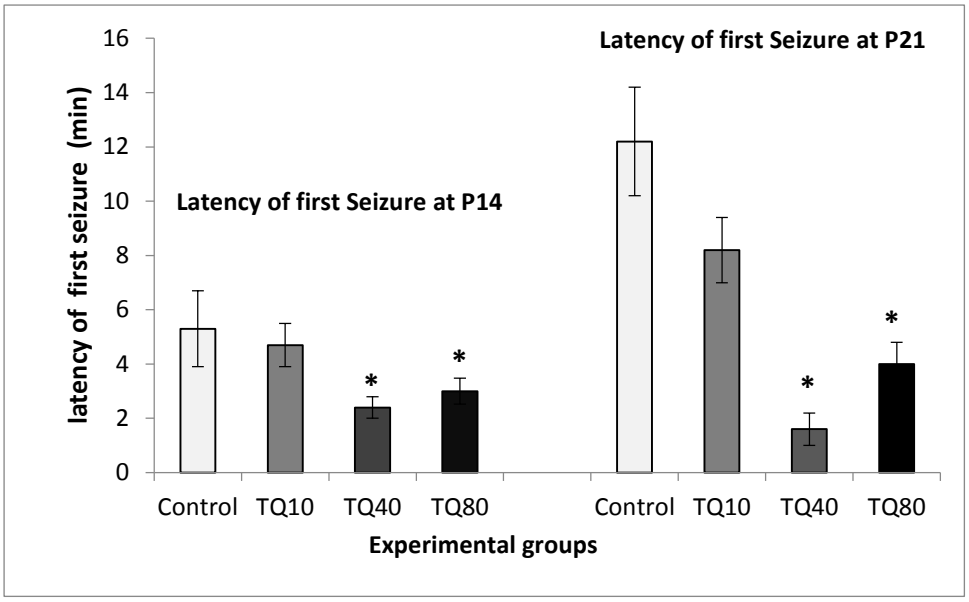

Fig.4. Letancy to first sizure at 14 and 21 days after birth. The values show mean±SEM, TQ10 (thymoquinone 10mg/kg), TQ40 (thymoquinone 40mg/kg) and TQ80 (thymoquinone 80mg/kg); *shows significance with TQ10 and control groups (*P*<0.01, Kruskal–Wallis).

Table1: Generalized seizure properties in rat pups that their dams treated with thymoquinone in preconception period.

Groups ⟶	control	TQ 10mg/kg	TQ40	TQ80	*P*-value
TC number P14	2.25 ± 0.48	2.38+ 0.50	0.44 ± 0.13*	0.31 ± 0.12*	**P*<0.003 VS Control and TQ10
TC duration P14 (second)	46.31± 10.5	40.94 ±8.33	14.19 ± 4.43*	8.0 ±3.39*	**P*<0.01 VS Control and TQ10
TC latency P14 (min)	15.62 ±2.8	17.12 ±2.2.54	43.56 ±5.34*	51.44 ±3.91*	**P*<0.001 VS Control and TQ10
TC number P21	2.62± 0.55	3.0 ± 0.52	0.75 ± 0.17#	0.5 ± 0.13#	# *P*< 0.007 VS control and TQ10
TC duration P21(second)	57.62 ± 10.7	49.81 ±7.88	18.19 ± 4.43#	10.19 ± 2.84#	# *P*< 0.001 VS control and TQ10
TC latency P21(min)	17.0 ±2.4	18.94 ± 2.4	39.94 ±5.66#	46.62 ± 3.92#	# *P*< 0.001 VS control and TQ10

TC: tonic-clonic seizure, TQ: thymoquinone; Data were analyzed by Kruskal–Wallis and/or Mann-Whitney U tests)

TQ was significant (F(3,44)=23.74, *P*<0.001) and effect of age was not significant (F(1,44)=0.01, *P*=0.984). Interaction of TQ and age was not significant (F(3,44)=0.91, *P*=0.443). Pre-gestational administration of TQ (80mg/kg) significantly decreased seizure stages in pups at P14 and P21 (*P*<0.001), as shown in Figures 2. The other doses of TQ (10 and 40mg/kg) had no significant effect on seizure stages. Although at the P21, the 40mg/kg also decreased seizure stages over time, this decrease was not statistically significant. Pre-gestational administration of higher TQ dose prevents the progress of seizure to a more complex generalized seizure in pups.

Effect of pre-gestational administration of TQ on focal seizure duration in rat offspring

Data related to focal seizure duration were analyzed by Kruskal–Wallis and/or Mann-Whitney U tests. Pre-gestational administration of 40 and 80mg/kg TQ significantly increased focal seizure duration compared to the control group at P14 (*P*<0.05). At

P21, only TQ 80mg/kg had significant effect on seizure duration (Fig. 3).

Effect of pre-gestational administration of TQ on latency to first seizure in rat offspring

Pre-gestational administration of TQ (40 and 80mg/kg) significantly decreased latency to first seizure in PTZ-induced seizure in rat offspring at P14 and P21 (P<0.01), as shown in Figure 4. TQ facilitated the initiation of focal seizures and prevented the development of more complex generalized seizure.

Effect of pre-gestational administration of TQ on tonic-clonic seizure in rat offspring

As shown in Table 1, the pre-conceptional administration of 40 and 80mg/kg TQ significantly attenuated the tonic-clonic seizure compared to the control group at P14 and P21. Moreover, TQ 10mg/kg had no significant effects on tonic-clonic seizure.

Discussion

In the current study, female Wistar rats were treated with different doses of TQ prior to conception; then, the offspring was assessed in terms of PTZ-induced seizure. The result revealed that TQ before gestation suppressed the seizure progress and prevented the rats to reach upper stages of seizure, while it potentiated the induction of focal seizure by decreasing latency to first seizure behavior and increasing the duration of focal seizures (short myoclonic jerk) induced by PTZ in offspring. Meanwhile, the effect of age was insignificant as revealed by two-way ANOVA data analysis.

The neuroprotective effects of NS oil and its major constituent, TQ, have been studied in many brain insulting events including epilepsy (Farkhondeh et al., 2018). NS oil shows anticonvulsant and antioxidant effects against PTZ-induced kindling in mice more potently than valproate (Ilhan et al., 2005). Likewise, the aqueous extract of this plant suppresses penicillin-induced epileptic activity in rats. This anticonvulsant effect is a consequence of selective monoamine level altering in different brain regions (Guha et al., 2005). Previous studies supported the hypothesis indicating that the sedative and depressive effects of NS observed *in vivo* could be based on the changes of inhibitory/excitatory amino acid levels (El-Naggar et al., 2010; Noor et al., 2012). The neuroprotective effect of NS on amino acid neurotransmitters alteration in PTZ and ciprofloxacin (CFX) treated rats in different brain regions was examined in a study. The oral administration of NS induced an elevation in aspartate and glutamate contents, whereas the GABA and glycine levels were decreased. Furthermore, the treatment with PTZ and CFX caused a decrease in aspartate, glutamate and total antioxidant capacity levels, while the GABA and glycine concentrations were increased after 14 days. Moreover, the pre- and post-treatment with NS in PTZ and CFX treated rats return the levels of these parameters to the control values. It was thus concluded that the treatment with CFX induced an imbalance in the excitatory and the inhibitory amino acids, which may lead to the initiation of epileptic seizures and that the treatment with NS ameliorated these neurological defects, indicating its potent antiepileptic activity (Arafa et al., 2013). In a study, the effects of the aqueous extract of NS on PTZ-induced seizure model were evaluated among the adult rats. The effects of the extract on seizure parameters such as ictal phase duration, interictal phase duration, seizure score and EEG were studied. The rats receiving pre-treatment with NS extract showed much more resistance to convulsion than the control rats. Picrotoxin, a GABAA antagonist, antagonized the reduction of seizure activity by NS. Hence the findings suggest that the NS may have an anticonvulsant activity in the petitmal epilepsy through an increase in GABAergic tone (Debasis Biswas and Guha, 2007).

TQ is the major constituent of NS seeds and the anticonvulsant activity of this compound was evaluated in the experimental and clinical studies. It has been reported that 40 and 80mg/kg TQ prolong the onset of seizures and reduce the duration of myoclonic seizures induced by PTZ but not by maximal electroshock model. The complete protective effect against mortality, however, was reported in both models (Hosseinzadeh and Parvardeh, 2004). In a lithium–pilocarpine rat model of status epilepticus, it was indicated that TQ decreased brain damages induced by status epilepticus via modulating the nuclear factor erythroid 2–related factor 2 (Nrf2) signaling pathway involved in the activation of the antioxidant defense system

(Shao et al., 2017). Also, it has been reported that TQ attenuated epilepsy by declining gene expression of NF-κB, which mediates inflammatory reactions. TQ improved electroencephalography profiles, lowered death rate, decreased seizure severity and improved learning and memory functions in experimental seizure models (Farkhondeh et al., 2018). Protective effect of TQ on temporal lobe epilepsy (TLE) has also been reported; in the intrahippocampal kainate model of TLE in rat, TQ decreased oxidative stress indices and nitrate in the hippocampal tissue and severe seizure activity. It also improved astrogliosis and reduction in neurons in CA1, CA3, the hilar regions and mossy fiber sprouting in the dentate gyrus of kainate-lesioned rats (Dariani et al., 2013).

Moreover, the anticonvulsant effect of TQ was evaluated by intracerebroventricular (ICV) injection using PTZ-induced seizure model. The ICV administration of TQ prolonged the onset and reduced the duration of tonic-clonic seizures. Flumazenil (1nmol, ICV) reversed the anticonvulsant activity of TQ. Further, the pretreatment with naloxone (10µmol, ICV) antagonized the prolongation of tonic-clonic seizure latency as well as the reduction of seizure duration induced by TQ (ICV). Thus the TQ may possess anticonvulsant activity, which might be the result of an opioid receptor-mediated increase in GABAergic tone (Hosseinzadeh et al., 2005). A double-blinded crossover clinical trial study of TQ (1mg/kg) as an adjunctive therapy was performed on children with refractory epilepsy and its effects on the frequency of seizures were compared with those of a placebo. The patients were assigned into two groups and received either TQ or placebo for a period of four weeks. Then, they received only their preexisting antiepileptic drugs during the two weeks of washout period; then, after cross-overing, they received the TQ or placebo for a period of four weeks again. Throughout these periods, their effects on seizure frequency were investigated. The reduction of frequency of seizures at the end of first period in comparison to the same period prior to the study demonstrated a significant difference between the two groups. (Akhondian et al., 2011). In the above-mentioned studies, TQ showed anti-seizure and/or anticonvulsant properties and prolonged the onset and reduced the duration of tonic-clonic seizures. The finding of current study is consistent with these literatures. Meanwhile, we could not find any reports regarding focal seizure potentiation by TQ neither agreeing nor opposing documents. We suggest conduction of more accurate study focusing on focal and generalized seizure effects of TQ directly on subjects (not on offspring).

An important difference between the current study and the above-mentioned studies is the time when the TQ was used. In all of these studies, TQ has been directly applied on the subjects and its anticonvulsant effects were evaluated on the same subjects; however, in the current study exposure to the TQ before conception and its anticonvulsant properties were studied in the next generation (offspring). Although changes in body of rats receiving TQ such as alterations in hormones and enzymes in reproductive system (and other organs) can influence the seizure susceptibility in offspring, the effects mainly seem to be transferred to the offspring through the epigenetic pathway. An example can be alteration in activity of 11β-hydroxysteroid dehydrogenase in uterus of TQ-treated mothers which regulates the level of steroid exposure of fetus in normal and stress conditions (Tomlinson and Stewart, 2001). Previous studies on pre-gestational stress revealed that environmental conditions can be transferred to the next generation by means of epigenetics factors and parental gametes (Mahmoodkhani et al., 2018). Thus, feeding with TQ prior to conception can seemingly make changes in oocyte environment and transfer to the offspring. It is well-established that oocyte quality determines the embryo's developmental potential and even adult's disease after fertilization (Krisher, 2004; Miao et al., 2009). Critical events of oogenesis occur during three distinct developmental stages: meiotic initiation in the fetal ovary, follicle formation in the perinatal period and oocyte growth as well as maturation among the adults. Recent experimental studies suggest that environmental influences might adversely affect all of the three stages. The changes during the final stages of oocyte growth may have a more severe impact on the genetic quality of the oocyte (Hunt and Hassold, 2008; Mahmoodkhani et al., 2018).

Unfortunately, no study is available on the effects of pre-gestational administration of TQ in offspring; therefore, comparing the results of the present study with the previous studies is difficult. It is not clear how TQ affects the mothers in general and their oocytes

particularly. This study seems to be the first of its kind and can shed a light on this field. Therefore, its results are unique, even though, caution is required in interpreting the findings. In addition, the results of present study showed that high dose of TQ increased pups body weight. These results are consistent with Faisal's et al. results suggesting that Sprague-Dawley rats regained their weight following TQ administration in pristine-induced arthritis (Faisal et al., 2015).

Conclusion

It can be concluded that supplementation of TQ before conception may have biphasic effects on seizure in offspring. It seems that TQ suppresses seizure progress and prevents development of generalized seizure, while it potentiates focal seizures initiation and development in rat offspring during infancy. Although we suggest hormonal and enzymatic alterations in mother and epigenetic factors in oocyte for underlying mechanism of these findings, further studies need to clarify the various aspects of the TQ effect before conception in offspring.

Acknowledgments

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Conflict of interest

Authors declare no conflict of interest regarding this paper.

References

Abdel-Rahman M, Arafa NM, El-khadragy MF, Kassab RB. The neuroprotective role of nigella sativa extract on ciprofloxacin and pentylenetetrazole treated rats. Afr J Pharm Pharmacol 2013; 7: 1660-1670.

Akhondian J, Kianifar H, Raoofziaee M, Moayedpour A, Toosi MB, Khajedaluee M. The effect of thymoquinone on intractable pediatric seizures (pilot study). Epilepsy Res 2011; 93: 39-43.

Akhondian J, Parsa A, Rakhshande H. The effect of nigella sativa l. (black cumin seed) on intractable pediatric seizures. Med Sci Monit 2007; 13: Cr555-9.

Arafa NM, Abdel-Rahman M, El-khadragy MF, Kassab RB. Evaluation of the possible epileptogenic activity of ciprofloxacin: the role of nigella sativa on amino acids neurotransmitters. Neurochem Res 2013; 38: 174-185.

Bloomfield FH, Oliver MH, Hawkins P, Holloway AC, Campbell M, Gluckman PD, et al. Periconceptional undernutrition in sheep accelerates maturation of the fetal hypothalamic-pituitary-adrenal axis in late gestation. Endocrinology 2004; 145: 4278-4285.

Dariani S, Baluchnejadmojarad T, Roghani M. Thymoquinone attenuates astrogliosis, neurodegeneration, mossy fiber sprouting, and oxidative stress in a model of temporal lobe epilepsy. J Mol Neurosci 2013; 51: 679-86.

Debasis Biswas, Guha D. Nigella sativa: its role as an anticonvulsant in pentylenetetrazole induced seizures. Biogenic Amines 2007; 21: 66-76.

Ebisch I, Thomas C, Peters W, Braat D, Steegers-Theunissen R. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. Hum Reprod Update 2006; 13: 163-174.

El-Naggar T, Gómez-Serranillos MP, Palomino OM, Arce C, Carretero ME. Nigella sativa l. Seed extract modulates the neurotransmitter amino acids release in cultured neurons in vitro. Biomed Res Int 2010; 2010.

Faisal R, Chiragh S, Popalzai AJ, Rehman KU. Anti inflammatory effect of thymoquinone in comparison with methotrexate on pristane induced arthritis in rats. J Pak Med Assoc 2015; 65: 519-25.

Farkhondeh T, Samarghandian S, Shahri AMP, Samini F. The neuroprotective effects of thymoquinone: a review. Dose-response 2018; 16: 1559325818761455.

Guha D, Biswas D, Purkayastha S. Suppression of penicillin-induced epileptiform activity by nigella sativa: Possible mediation by neurotransmitters. Biogenic Amines 2005; 19: 309-322.

Heidari M, Dadollahi Z, Mehrabani M, Mehrabi H, Pourzadeh-Hosseini M, Behravan E, et al. Study of antiseizure effects of matricaria recutita extract in mice. Ann N Y Acad Sci 2009; 1171: 300-304.

Hosseinzadeh H, Khosravan V. Anticonvulsant effects of aqueous and ethanolic extracts of crocus sativus l stigmas in mice. Arch Iran Med 2002; 5: 44-47.

Hosseinzadeh H, Madanifard M. Anticonvulsant effects of coriandrum sativum l. Seed extracts in mice. Arch Iran Med 2000; 3: 81-84.

Hosseinzadeh H, Parvardeh S. Anticonvulsant effects of thymoquinone, the major constituent of nigella sativa seeds, in mice. Phytomedicine 2004; 11: 56-64.

Hosseinzadeh H, Parvardeh S, Nassiri-Asl M, Mansouri M-T. Intracerebroventricular administration of thymoquinone, the major constituent of nigella sativa seeds, suppresses epileptic seizures in rats. Med Sci Monit 2005; 11: BR106-BR110.

Hunt PA, Hassold TJ. Human female meiosis: what makes a good egg go bad? Trends Genet 2008; 24: 86-93.

Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M. Antiepileptogenic and antioxidant effects of nigella sativa oil against pentylenetetrazol-induced kindling in mice. Neuropharmacology 2005; 49: 456-464.

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: 1693-1705.

Krisher R. The effect of oocyte quality on development. J Anim Sci 2004; 82: E14-E23.

Mahmoodkhani M, Saboory E, Roshan-Milani S, Azizi N, Karimipour M, Rasmi Y, et al. Pregestational stress attenuated fertility rate in dams and increased seizure susceptibility in offspring. Epilepsy Behav 2018; 79: 174-179.

Miao Y-L, Kikuchi K, Sun Q-Y, Schatten H. Oocyte aging: cellular and molecular changes, developmental potential and reversal possibility. Hum Reprod Update 2009; 15: 573-585.

Mostafa RM. Potential antiepileptic therapeutic effect of nigella sativa: a new chapter in the old story of epilepsy. Spatula DD 2013; 3: 31-32.

Noor NA, Ezz HSA, Faraag AR, Khadrawy YA. Evaluation of the antiepileptic effect of curcumin and nigella sativa oil in the pilocarpine model of epilepsy in comparison with valproate. Epilepsy Behav 2012; 24: 199-206.

Rahmati B, Khalili M, Roghani M, Ahghari P. Anti-epileptogenic and antioxidant effect of lavandula officinalis aerial part extract against pentylenetetrazol-induced kindling in male mice. J Ethnopharmacol 2013; 148: 152-157.

Raza M, Alghasham AA, Alorainy MS, El-Hadiyah TM. Potentiation of valproate-induced anticonvulsant response by nigella sativa seed constituents: the role of gaba receptors. Int J Health Sci 2008; 2: 15-25.

Salarinia R, Rakhshandeh H, Oliaee D, Gul Ghasemi S, Ghorbani A. Safety evaluation of phytovagex, a pessary formulation of nigella sativa, on pregnant rats. Avicenna J Phytomed 2016; 6: 117-123.

Schoenaker DA, Soedamah-Muthu SS, Callaway LK, Mishra GD. Prepregnancy dietary patterns and risk of developing hypertensive disorders of pregnancy: results from the australian longitudinal study on women's health. Am J Clin Nutr 2015; 102: 94-101.

Shao YY, Li B, Huang YM, Luo Q, Xie YM, Chen YH. Thymoquinone attenuates brain injury via an anti-oxidative pathway in a status epilepticus rat model. Transl Neurosci 2017; 8: 9-14.

Sousa DoPd, NÃ³brega FF, Santos CC, Benedito RB, Vieira YW, Uliana MP, et al. Anticonvulsant activity of thymoquinone and its structural analogues. Rev bras farmacogn 2011; 21: 427-431.

Tomlinson JW, Stewart PM. Cortisol metabolism and the role of 11beta-hydroxysteroid dehydrogenase. Best Pract Res Clin Endocrinol Metab 2001; 15: 61-78.

Vilela AA, Farias DR, Eshriqui I, Vaz JdS, Franco-Sena AB, Castro MBT, et al. Prepregnancy healthy dietary pattern is inversely associated with depressive symptoms among pregnant brazilian women J Nutr 2014; 144: 1612-1618.