RESEARCH ARTICLE

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Evaluation of the Protective Effect of the Cup Therapy on the Epileptic Seizure in Rats

ABSTRACT

Objective: Cup therapy has an important place in traditional and complementary medicine applications. The purpose of our study, this is the first time to investigate the protective effect of cup therapy in rats on experimentally generated epileptic seizures in new rat modeling created by different anatomic regions.

Methods: A total of 42 Wistar albino rats were randomly divided into 6 groups (n:7). The "new dry cup" was applied to the G1 group, and the "new wet cup" model was applied to the G2. In the G3 group, an "epilepsy model" (PTZ, 35 mg / kg) was created and "diazepam" (2.5 mg / kg) was given to G4. "Dry cup" and "wet cup" models were applied to the G5 and G6 groups, respectively. 24 hours after the cupping therapies, the rats were injected with PTZ and the epilepsy behavior scores of the rats in all groups were recorded for 20-30 minutes.

Results: In the 'Open Area' and 'Elevated Plus Maze' tests, there was no behavioral difference between the cup therapy group and the control group ($p > 0.05$). Given all the parameters, the G4 group significantly reduces the seizure compared to other groups ($p < 0.05$). There is a significant difference in G2, G5 and G6 groups compared to G3 in the phases parameter ($p < 0.05$).

Conclusions: In this study, the new wet cup therapy (G2), which was applied for the first time, had a protective effect on seizures. G2, G5 and G6 groups are observed to suppress seizures compared to G3. Our findings are expected to contribute greatly to animal model analysis in the future.

Keywords: Epilepsy, Seizure, Cup Therapy, Hijama, Rat, Animal Model.

Kupa Terapisinin Ratlarda İndüklenen Epileptik Nöbet Üzerine Koruyucu Etkisinin Değerlendirilmesi

ÖZET

Amaç: Geleneksel ve tamamlayıcı tıp uygulamaları içerisinde kupa terapisi önemli bir yer almaktadır. Çalışmamızın amacı, ilk kez sıçanlarda kupa terapisinin farklı anatomik bölge tayini ile oluşturulan yeni rat modellemesinde, deneysel olarak oluşturulmuş epileptik nöbetler üzerine koruyucu etkisinin araştırılmasıdır.

Gereç ve Yöntem: Çalışmamızda, 42 adet Wistar albino cinsi 3-4 aylık (200-250 gr) sıçanlar kullanılmıştır. G3 ve G4 dışındaki diğer dört gruba (n=7), kupa terapisi uygulamasından 24 saat sonra, PTZ (35 mg/kg, sc., 0,2 cc) enjekte edildi ve ardından davranışsal epilepsi skorlaması yapılmıştır.

Bulgular: Toplamda 42 adet Wistar albino sıçan rastgele 6 gruba (n:7) ayrılmıştır. G1 grubuna "yeni kuru kupa", G2'ye ise "yeni yaş kupa" modeli uygulanmıştır. G3 grubunda, "epilepsi modeli" (PTZ, 35 mg/kg) oluşturulmuş ve G4'e diazepam (2,5 mg/kg) verilmiştir. G5 ve G6 gruplarına ise "kuru kupa" ve " yaş kupa " modelleri sırasıyla uygulanmıştır. Kupa terapilerinden 24 saat sonra, sıçanlara PTZ enjekte edilmiş ve daha sonra tüm gruplardaki sıçanların epilepsi davranış skorları 20-30 dakika boyunca kaydedilmiştir.

Sonuç: Bu çalışmada, ilk defa uygulanmış olan yeni yaş kupa terapisinin (G2) nöbetler üzerine koruyucu etkisi bulunmuştur. G2, G5 ve G6 gruplarının G3'e kıyasla nöbeti baskıladığı gözlemlenmektedir. Bulgularımızın ileride bu konuda yapılacak hayvan modeli analizlerine büyük ölçüde katkı sağlaması beklenmektedir.

Anahtar Kelimeler: Epilepsi, Nöbet, Kupa Terapisi, Hacamat, Rat, Hayvan Modeli.

INTRODUCTION

Epilepsy is a progressive neurodegenerative disease characterized by recurrent seizures as a result of abnormal and synchronous neuron hyperactivity due to multifactorial causes. It was observed that the incidence of epilepsy was higher in childhood and old age, and it was at a lower level in early adulthood (1, 2). According to the International Association for Combating Epilepsy (ILAE) criteria, the diagnosis of idiopathic epilepsy is seen in the rate of 36-44 / 1000 (3). According to clinical information and electroencephalography (EEG) changes, no underlying pathological process was detected in idiopathic epilepsies (4).

Pentylenetetrazole (PTZ) induced animal model is widely used in the investigation of epilepsy pathophysiology. It is known to be effective by binding to γ aminobutyric acid type A (GABA-A) receptors associated with the postsynaptic Cl- channels, which are the binding site of picrotoxin in general. In the message regulated with N-Methyl-D aspartate (NMDA) receptors, it plays an important role in the formation of PTZ-induced generalized tonic-clonic seizures (6).

Traditional and complementary medicine (TCM) includes 14 different types of application areas within the scope of the Ministry of Health. It is used in the treatment of many diseases such as asthma, cellulite, fibromyalgia, hypertension, ischemia and allergic rhinitis, as well as in pathological conditions affecting the nervous system (7, 8). Cup therapy is an important part of TCM applications.

Cup therapy has been widely used in many regions of the world for centuries in the diagnosis and treatment of different diseases (9, 10). Although there are many variations of cup therapy, there are two most used forms. The first of these is a dry cup treatment known as “cupping” and there is no incision in this method and no blood is drawn after cupping (11). In the second cugging method known as Hijama, after the incision is applied to certain points in the body, the blood is released by creating negative pressure with the cup. In the literature, there are studies evaluating the use of wet cup therapy in diseases affecting the nervous system, such as migraine (12), neuralgia, manic depression and mental diseases (13), as well as in patients with epilepsy (14). Although there are many publications in the literature evaluating the application of cup therapy on humans, studies in experimental rat models are limited. In addition, no study investigating the effects of cup therapy has been found in experimentally created epilepsy animal models.

In a study by Subadi et al., cup therapy was applied only in the paralumbar region (15). However, the regions selected for cup therapy applications in neurodegenerative diseases such as migraine in humans; cervical 7, thoracic 3 interscapular region, acupuncture areas such as thoracic 7. Our study, lack of an effective cup therapy model in the literature in experimental studies on neurodegenerative diseases and it is important in terms of understanding stress-related processes in the application.

Therefore, the aim of our study is to investigate the protective effect of experimentally generated epileptic seizures in the new rat modeling created by different anatomic region determination of cup therapy in rats.

MATERIAL AND METHODS

Experimental Animals: In the study, 3-4 months old (200-250 g) rats of Wistar albino type were used. The experimental animals used in the study were obtained from the Bolu Abant Izzet Baysal University Experimental Animals Application Research Center. The animals were fed ad libitum in type IV cages for 12 hours in a light / dark environment with a relative humidity of 55-60%. Each group was determined to have 7 rats. Accordingly, a total of 42 rats were divided into 6 groups (Figure 1).

Experimental Design: Before applying dry and wet cup therapy, 90/10 mg/kg intamuscular ketamine/xylazine anesthesia was applied to each rat (Figure 2).

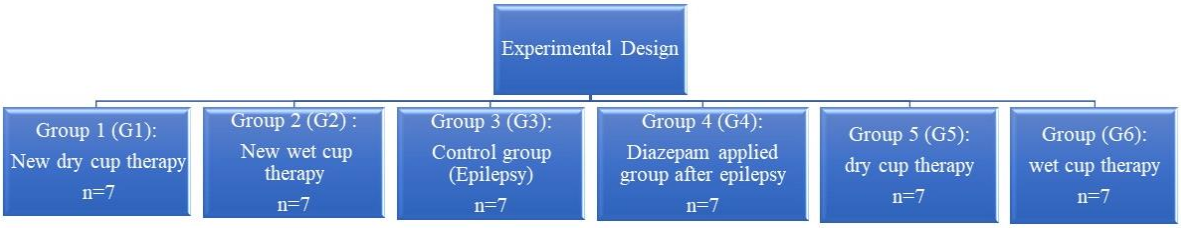

Figure 1. Experimental Groups

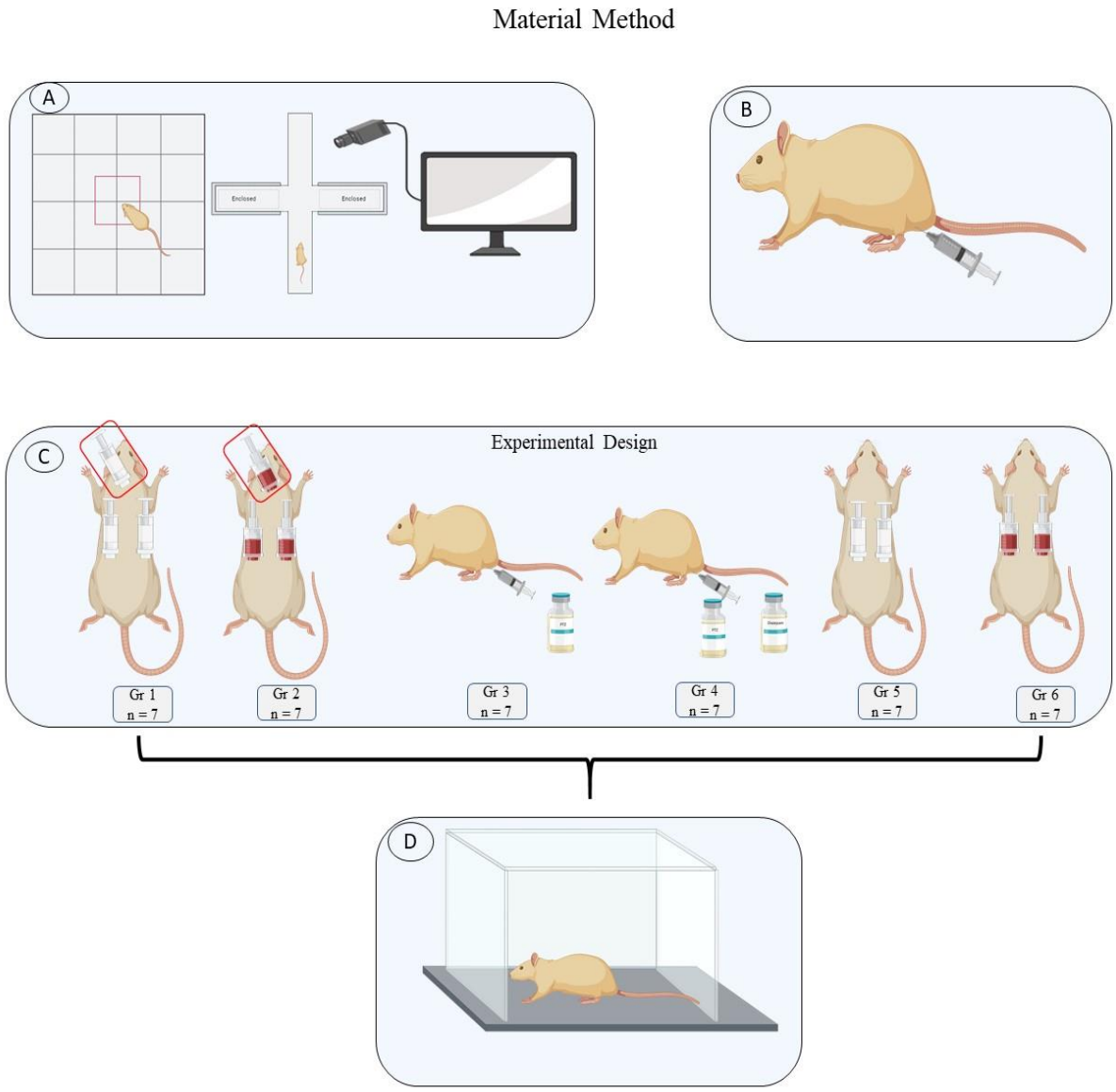

Figure 2. Material Methods
A: 'Open Field' and 'Elevated Plus Maze' Tests. **B:** Animals were anesthetized intramuscular (im) with 90 mg/kg ketamine and 10 mg/kg xylazine.**C:** Experimental Design (New dry cup therapy, New wet cup therapy, Control, Diazepam applied group after epilepsy, dry cup therapy, wet cup therapy, respectively). **D:** After epilepsy modeling, the behavior of the subjects was recorded with a camera for 20 minutes. Three measurement methods were used to evaluate the seizures: (1) Racine's Convulsion Scale (RCS), latency times of 'first myoclonic jerk' (FMJ), latency times of 'first generalized seizure' (FGS)

Cup Therapy Models:

Wet Cup Therapy: According to the method made by Subadi et al. (15), the skin of the left and right paralumbar regions of the G6 rats were punctured with a lancet, and then sterile cups (1 cm in diameter) were placed and negative pressure (-200 mm Hg) was applied for 5 minutes (Figure 3-A).

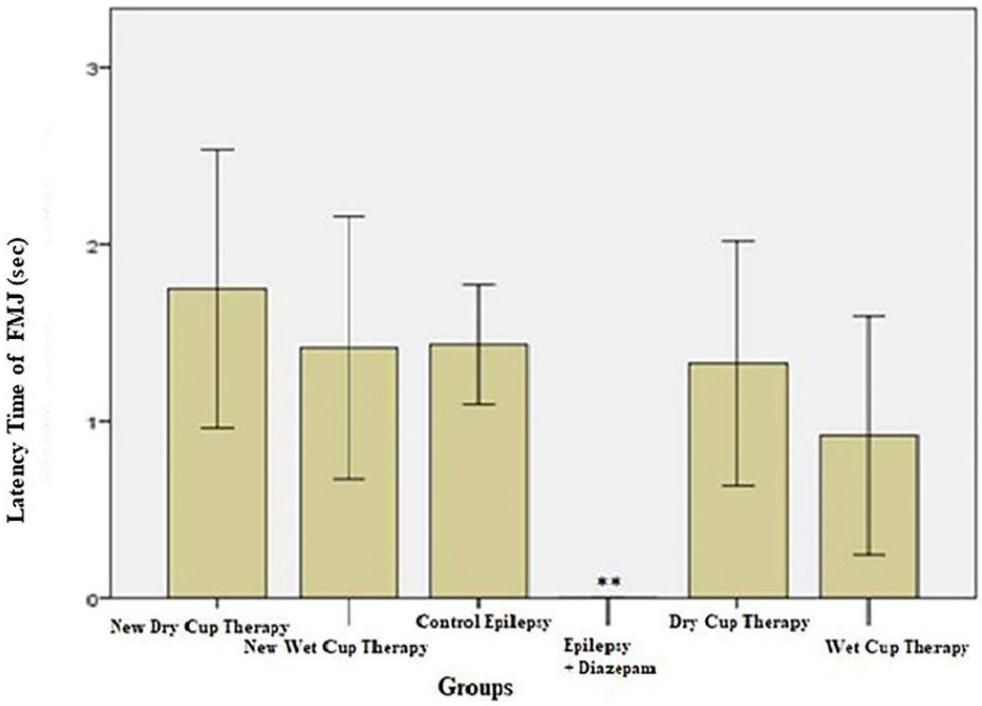

Figure 3. Latency Time of 'First Myoclonic Jerk' (FMJ) (sec)

Dry Cup Therapy: According to the method made by Subadi et al. (15), sterile cups (1 cm in diameter) were placed in to the skin of the left and right paralumbar regions of the G5 rats and negative pressure (-200 mm Hg) was applied for 5 minutes.

New Cup Therapy Models:

New Wet Cup Therapy: In addition to the left and right paralumbar areas made by Subadi et al. (15), the skin of the cervical 7 (C7) region of the G2 rats were also punctured with lancet, sterile cups (1 cm in diameter) were placed and then, negative pressure (-200 mm Hg) was applied for 5 minutes (Figure 3-B).

New Dry Cup Therapy: In addition to the left and right paralumbar areas made by Subadi et al. (15), sterile cups (1 cm in diameter) were also placed in to the skin of the cervical 7 (C7) regions of the G1 rats and negative pressure (-200 mm Hg) was applied for 5 minutes.

Animal Model of Epilepsy and Behavior Scoring: PTZ used in epilepsy modeling was provided by P6500-Sigma-Aldrich. PTZ (35 mg/kg or 3.5mg/mL) was prepared by dissolving in a volume of 10 ml/kg in 0.9 % physiological salt solution (16). Epilepsy modeling was performed by subcutaneous injection of 0.2 cc for each subject from this prepared solution (17). 24 hours after cup therapy applications to G1, G2, G5 and G6 groups, PTZ injections were made. After epilepsy modeling, the behavior of the subjects was recorded with a camera for 20 minutes.

Three measurement methods were used to evaluate the seizures:

(1) Racine's Convulsion Scale (RCS) (18) (Table 1),

(2) latency times of 'first myoclonic jerk' (FMJ) (19)

(3) latency times of 'first generalized seizure' (FGS) (20).

In this experiment, the time to get at least 3 points represents the rat's FMJ (19) and the time to get at least 4 points represents FGS (20).

Table 1. Racine's Convulsion Scale (RCS) (18)

0	No Seizure
1	Twitching of vibrassae and pinnae
2	Motor arrest with more pronounced twitch
3	Motor arrest with generalized myoclonic jerks
4	Tonic clonic seizure while remaining on animal feed
5	Tonic -clonic seizure with loss of correction reflex
6	Fatal seizure witching of vibrasse and pinnae

Statistical Analysis: Statistical analysis of all groups were carried out using the SPSS (version.20) program. The statistical significance was assessed by one-way ANOVA and $p < 0.05$ values were considered statistically significant.

RESULTS

'Open Field' and 'Elevated Plus Maze' Tests: Before studying the effect of cup therapy on epileptic seizures, different groups have been formed and whether the cup therapy applications cause stress or not has been evaluated in the 'Open Field' and 'Elevated Plus Maze' tests. No behavioral difference was found between the cup therapy (incision+cupping) and control (non cup therapy) ($p > 0.05$).

Anti-epileptic Effects of Cup Therapy: Compared to other groups, we showed that the diazepam group significantly reduced PTZ-induced epileptic seizures in rats, with a greater decrease in mean RCS, and greater prolongation in FMJ and FGS, ($p < 0.05$) (Fig. 3,4). On the other hand, there is a decrease in means of RCS in G2, G5 and G6 groups compared to G3 ($p < 0.05$) (Fig. 5). There was no statistically significant difference between the other groups in terms of antiepileptic effects ($p > 0.05$).

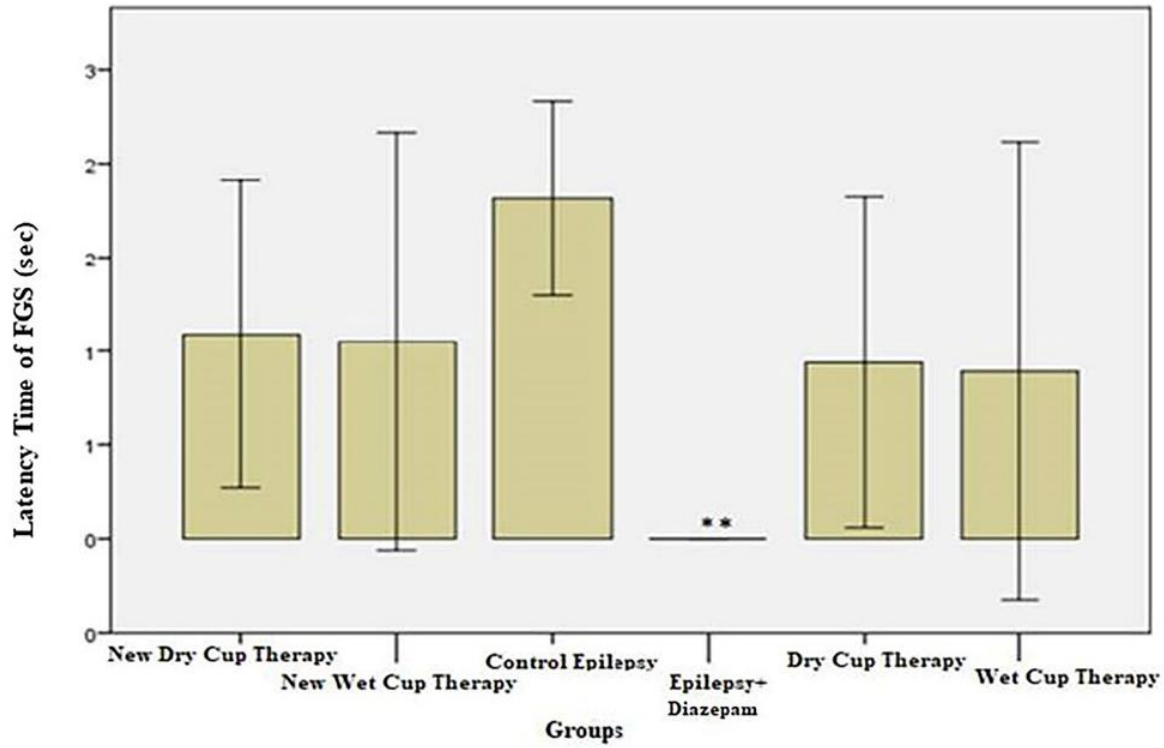

Figure 4. Latency Times of 'First Generalized Seizure' (FGS) (sec)

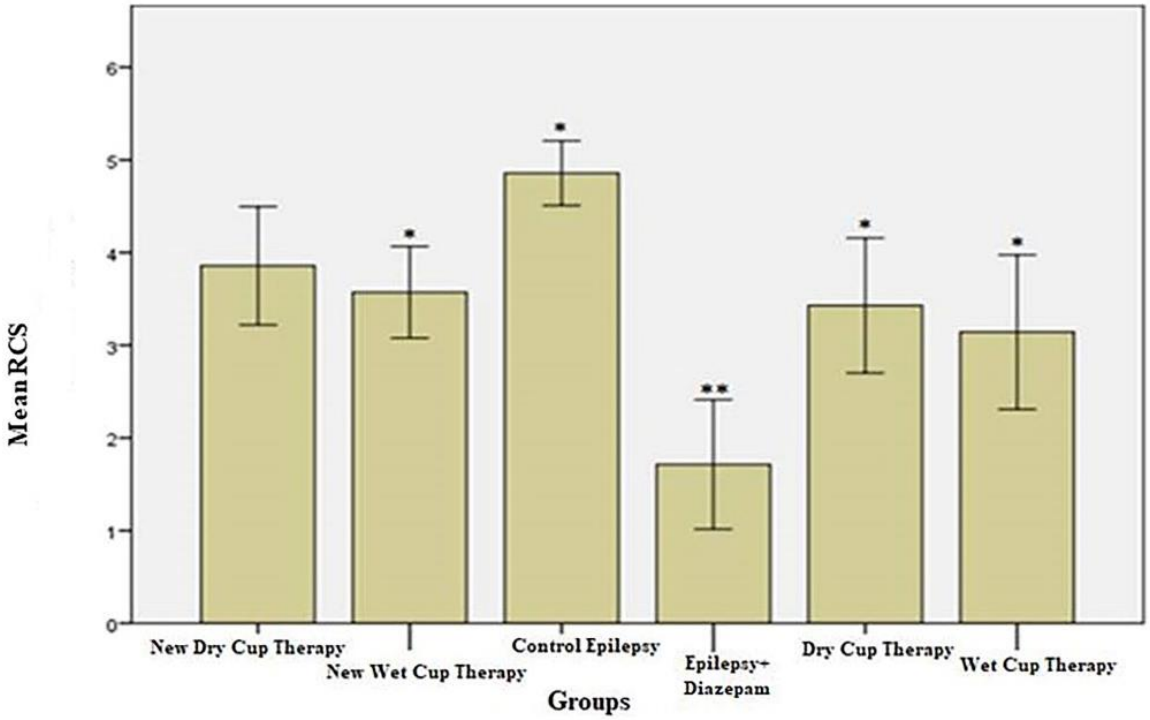

Figure 5. Mean Racine's Convulsion Scale (RCS)

DISCUSSION

Cup therapy is a very old method used as a complementary treatment in many diseases in the world (21). The history of the cup theory in China goes back over 2000 years, and its various versions have been used in parts of India, Arabia, Europe and Africa (22). It is used for many conditions such as infections, pain, mental disorders, heart diseases, various common diseases and skin diseases (21). It is known to be used in high blood pressure, especially tension type headaches, migraine, musculoskeletal pain, asthma, stroke, acne and some rheumatic diseases. In addition, cup therapy is recommended for pain and paralysis, stroke rehabilitation and complications, and neurological diseases such as Parkinson's disease (23). Eghbalian et al. from Iran, reported that wet cup therapy can be used in the treatment of epilepsy by applying it to the back of both legs (24). In the treatment of epilepsy, wet cup is used in the cervical, shoulder, leg and occipital regions, and dry cup is used in the head and legs (21).

Although different theories have been proposed, in epilepsy treatment, the mechanism of action of cup therapy has not yet been elucidated by experimental evidence. In the national health survey covering 23,393 people in the USA, they compared individuals with neurological diseases (tension headache, migraine, back and leg pain, stroke, dementia, epilepsy, memory loss) to healthy individuals. It has been observed that the use of TCM is high in patients with neurological diseases (44.1% vs. 32.6%) (25). In another study, it was emphasized that cup therapy removes oxidants and reduces oxidative stress (26). In the literature, although there are many studies investigating the effectiveness of cup therapy in neurological diseases, including epilepsy in humans, the number of animal model studies in which cup therapy is applied is very low.

Many theories have been proposed to explain the various effects and mechanisms of action of cup therapy (27). Various researchers have proposed biological and mechanical processes associated with cup therapy. For example, pain reduction is caused by the biomechanical properties of the skin, which is explained by the "Pain-Gate theory" (28), "Common Pest Inhibitory Controls" (29) and "Reflex Zone Theory" (30). Reflex Zone Theory and Pain-Gate Theory draws attention especially for the effect mechanism of cup therapy related to the nervous system (27, 31). According to the Reflex Zone Theory, the connections with the internal organs in the relevant regions of the segments formed by the spinal nerves are known as the Cutivesceral/viscerocutaneous reflex. In any pathological situation, skin changes or pain may occur in this area with the signal to the relevant skin area. According to this mechanism, it has been suggested that it is possible to contribute to the treatment of the organ with cup therapy to be made on the part related to the diseased organ. According to the Pain-Gate Theory, the thick unmyelinated A delta fibers are stimulated through the post-vacuum incision created with wet cup therapy, and the entrance doors of the pain signals reaching the medulla spinalis with the C group thin myelinated nerve fibers in the substantia gelatinosa are closed. In addition, with the stimulation of mechanoreceptors, other pain stimulation is prevented over nociceptive afferent fibers and its transport is prevented (31,32).

In order to contribute to the mechanism of action, we designed an experimental study on rats related to epilepsy and cup therapy for the first time in the literature. According to the findings in our study, no significant result was obtained for the antiepileptic effects related to FMJ and FGS in groups other than the G4 group ($p > 0.05$). Compared to other groups, we showed that the diazepam group significantly reduced PTZ-induced epileptic seizures in rats, with a greater decrease in mean RCS, and greater prolongation in FMJ and FGS, ($p < 0.05$). Diazepam group has higher protection compared to all cup therapy models ($p < 0.05$). Strikingly, in RCS, G6, G5, and G2 appear to suppress seizures compared to G3. To put it more clearly, in dry cup, wet cup and new wet cup therapy model groups, cup therapy has a protective effect compared to the group that has epilepsy ($p < 0.05$), while the new dry cup therapy model does not have a protective effect on seizures ($p > 0.05$). In addition, there is no significant difference in other groups RCS parameter ($p > 0.05$) (Figure 2-4). With the exception of the new dry cup therapy modeling, the suppressive effect of cup therapy on all seizures is surprising. This can cause many different underlying causes. Between these two different modeling, there are existing differences in terms of anatomical zoning and dry cup therapy has a significant effect on seizures as much as wet cup therapy. It is noteworthy that dry cup therapy, which is applied only to paralumbar areas, has a protective effect on seizures as well as wet cup therapy and new wet cup therapy. Further studies are needed to clarify the existing differences in terms of anatomical regions in two different models. In addition, epigenetic and anatomical differences in animals may cause errors in statistical results. In this context, in order to clarify the causes of differences, peripheral blood analyzes and histopathological brain tissue studies should be examined in detail. It is thought that detailed investigations and meta analysis studies will shed light on further studies to be carried out in the future.

CONCLUSION

While there is a significant difference in the new wet cup therapy group compared to the epilepsy group, the new dry cup therapy group (G1) created with C7 in addition to the paralumbar

region does not have a protective effect compared to G3. As a result; In RCS, G2, G5 and G6 groups seem to suppress seizures compared to G3. However, there is no significant difference in RCS when other groups are compared among themselves. Compared to other groups, the diazepam (G4) group further extended FMJ and FGS, thereby demonstrating its protective efficacy on their seizures. However, there is no significant difference between FMJ and FGS parameters among other groups.

This study is the first to investigate the protective effect of newly created cup therapy modeling on experimental epilepsy rat modeling. Our findings are expected to contribute greatly to future studies.

REFERENCES

1. Zupec-Kania BA, Spellman E. An overview of the ketogenic diet for pediatric epilepsy. Nutr Clin Pract. 2009;23:589-596.
2. Bertram EH. Temporal lobe epilepsy: where do the seizures really begin? Epilepsy Behav. 2009;14(Suppl 1):32-37.
3. Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L et al. ILAE classification of the epilepsies: Position paper of the ILAE commission for classification and terminology. Epilepsia. 2017;58:512-21.
4. Camfield P, Camfield C. Incidence, prevalence and aetiology of seizures and epilepsy in children. Epileptic Disord. 2015;17:117-23.
5. Macdonald RL, Barker JL. Phenobarbital enhances GABA-mediated postsynaptic inhibition in cultured mammalian neurons. Trans Am Neurol Assoc. 1977;102;139-140.
6. Velisek L, Kusa R, Kuluvana M, Mares P. Exitatory amino acid antagonist and pentylenetetrazol-induced seizures during ontogenesis: I. The effects of 2-amino7phosphonoheptonate. Life Sci. 1990;46:1349-1357.
7. Silva J, Monge-Fuentes V, Gomes F, Lopes K, dos Anjos L, Campos G et al. Pharmacological Alternatives for the Treatment of Neurodegenerative Disorders: Wasp and Bee Venoms and Their Components as New Neuroactive Tools. Toxins (Basel). 2015;7:3179-209.
8. Çağlayan HZB, Ataoğlu EE, Kibaroğlu S. Nörolojide Geleneksel ve tamamlayıcı tıp uygulamalarının etkinliğinin değerlendirilmesi. Turk J Neurol. 2018;24(2).
9. Furhad S, Bokhari AA. Cupping Therapy. In StatPearls [Internet]. 2019.
10. Umar NK, Tursunbadalov S, Surgun S, Welcome MO, Dane S. The effects of wet cupping therapy on the blood levels of some heavy metals: a pilot study. J Acupunct Meridian Stud. 2018;11(6):375-379.
11. Yoo SS, Tausk F. Cupping: east meets west. Int J Dermatol. 2004;43(9):664-5.
12. Ahmadi A, Schwebel DC, Rezaei M. The efficacy of wet-cupping in the treatment of tension and migraine headache. The American Journal of Chinese Medicine. 2008;36(1):37-44.
13. Bamfarahnak H, Azizi A, Noorafshan A, Mohagheghzadeh A. A tale of Persian cupping therapy: 1001 potential applications and avenues for research. J. Complement. Med. Res. 2014;21(1):42-47.
14. Nayak S, Kumar PH. A scientific appraisal on hirudotherapy-an evolution in life style disorders. IJPR. 2015;5(10):217-221.
15. Subadi I, Nugraha B, Laswati H, Josomuljono H. Pain relief with wet cupping therapy in rats is mediated by heat shock protein 70 and ß-endorphin. Iran. J. Med. Sci. 2017;42(4):384
16. McCandless DW, FineSmith RB. Chemically induced models of seizures. In animal models of neurological disease, II (pp. 133-151). Humana Press; 1992
17. Dhir Ashish. Pentylenetetrazol (PTZ) Kindling Model of Epilepsy. Current protocols in neuroscience/editorial board, Jacqueline N. Crawley et al. Chapter 9. Unit9.27; 2012.
18. Lüttjohann A, Fabene PF, Luijtelaar G. A revised Racine's scale for PTZ-induced seizures in rats. Physıol Behav. 2009;98(5):579-586.
19. Erbaş O, Solmaz V, Aksoy D. Inhibitor effect of dexketoprofen in rat model of pentylenetetrazol-induced seizures. Neurol Res. 2015;37(12):1096-1101.
20. Kaputlu I, Uzbay T. L-NAME inhibits pentylenetetrazole and strychnine-induced seizures in mice. Brain Res. 1997;753:98-101.
21. Nimrouzi M, Mahbodi A, Jaladat AM, Sadeghfard A, Zarshenas MM. Hijamat in traditional Persian medicine: risks and benefits. J Evid Based Complementary Altern Med. 2014;19(2): 128-136.
22. Wang Y, Lin XM, Zheng GQ. Traditional Chinese medicine for Parkinson's disease in China and beyond. J Altern Complement Med. 2011;17(5):385-8.
23. Wang Z, Wan H, Li J, Zhang H, Tian M. Molecular ımaging in traditional chinese medicine therapy for neurological diseases. Biomed Res Int. 2013;608430.
24. Eghbalian F, Kenari HM, Kordafshari G, Karimi M, Atyabi A, Shirbeigi L. The role of phlebotomy (fasd) and cupping in the treatment of epilepsy from perspective of Persian medicine. Iran J Public Health. 2019;48(7):1392-1394.
25. Wells RE, Phillips RS, Schachter SC, McCarthy EP. Complementary and alternative medicine use among U.S. Adults with common neurological conditions. J Neurol. 2010; 257(11):1822-1831.

26. Uddin SMM, Haq A, Sheikh H. The Use of hijama (wet cupping) in alternative and complementary medicine: efficacious or perilous?. J Acupunct Meridian Stud. 2016;9(6):285-286.
27. Bedah AMNA, Khalil MKM, Posadzki P, Sohaibani I, Aboushanab TS, AlQaed M et al. Evaluation of wet cupping therapy: systematic review of randomized clinical trials, J Altern Complement Med. 2016;22(10):768-777.
28. Moayedi M, Davis KD. Theories of pain: from specificity to gate control. J Neurophysiol. 2012;109:5-12.
29. Le Bars D, Villanueva L, Willer JC. Diffuse noxious inhibitory controls (DNIC) in animals and in man. Acupunct Med. 1991;9:47-56.
30. Ann Lett RM. Reflex Zone Therapy for Health Professionals. first ed. pp. 2–20. Amazon.com; 2000.
31. Sedat Y, Sevilay E, Nuray YP, Ümmü GY, Burcu BK, Yücel K et al. Geleneksel Çin Tıbbında bir şifa yöntemi -2 kupa terapisi için etki mekanizmaları: Derleme, Integr Tıp Derg. 2013;1(1):18-21. 20.
32. Musial F, Michalsen A, Dobos G. Functional chronic pain syndromes and naturopathic treatments: neurobiological foundations. Forsch Komplement Med 2008;15(2):97-10319.