Epilepsy Research 158 (2019) 106225

Contents lists available at ScienceDirect

Epilepsy Research

journal homepage: www.elsevier.com/locate/epilepsyres

Anticonvulsive and neuroprotective effects of aqueous and methanolic extracts of *Anacyclus pyrethrum* root in kainic acid-induced-status epilepticus in mice

Houria Manouze, Otmane Bouchatta, Mohamed Bennis, Zahra Sokar, Saâdia Ba-M'hamed*

Laboratory of Pharmacology, Neurobiology and Behavior, Cadi Ayyad University, Marrakesh, Morocco

ARTICLE INFO

Keywords:
Anticonvulsant
Neuroprotective
Anacyclus pyrethrum
Kainic acid
Mice

ABSTRACT

Purpose: Epilepsy is a chronic neurological disorder characterized by spontaneous and recurrent seizures. The currently available synthetic antiepileptic drugs have a limited efficacy and are associated with a wide range of side effects. In Ayurveda, *Anacyclus pyrethrum* root (APR) has been used as a traditional antiepileptic remedy. The aim of the present study is to evaluate the anticonvulsive and neuroprotective effects of aqueous and methanol extracts of *Anacyclus pyrethrum* root (AEAPR and MEAPR) on experimental model of status epilepticus (SE).

Methods: Twenty four male mice were divided into four groups. The control and KA groups had free access to tap water for 5 days before the intraperitoneal injection of distillated water or kainic acid (KA; 30 mg/kg), respectively. In the treated groups, mice received extracts solutions MEAPR and AEAPR in drinking water at the concentration of 5 g/l for 5 days. At the fifth day, animals received intraperitoneal injection of KA. The behavioral changes latency of seizures, the number of wet dog shakes (WDS) and the mortality were observed over 6 h. Thereafter, the mice were sacrificed for immunohistochemical studies.

Results: Pretreatment with MEAPR and AEAPR decreases significantly the frequency of WDS (32.5% and 43.9%, $p < 0.01$; respectively), and increases considerably the latent period (77.9% and 91.9%, $p < 0.01$; respectively) between the injection of the KA and the appearance of the SE as compared to the KA group. The duration and severity of seizure in the MEAPR or AEAPR-pretreated groups were significantly lower ($p < 0.01$ and $p < 0.05$ or $p < 0.01$; respectively) than those in the KA group. These behavioral results were confirmed by the immunohistochemical study at the level of the hippocampus, in which the c-FOS and GFAP expression of both MEAPR and AEAPR-treated animals largely reduced ($p < 0.001$) the number of labelled cells with respect to the group, which received the KA alone.

Conclusion: Our results showed that the MEAPR and AEAPR have anticonvulsive effect and putative neuroprotective effect against seizures induced by KA. Further studies are required to identify its active ingredients responsible for the observed effects.

1. Introduction

Epilepsy is the most common chronic neurological disease. It affects at least 65 million people worldwide and approximatively 80% of cases live in developing countries. It is characterized by recurrent unprovoked seizures (Thurman et al., 2011). Nearly 30% of people with epilepsy are resistant to currently available antiepileptic drugs (AEDs), which is related with increased risks of morbidity, injuries, psychosocial dysfunction, and an overall reduced quality of life (Kwan et al., 2011; Arroyo et al., 2002). Furthermore, the dose-related neurotoxicity, teratogenic effects and adverse effects on cognition and behavior associated with established AEDs limit their clinical use (Devinsky et al., 1995). The very high costs of the new AEDs have a major impact on the overall cost of epilepsy therapy in both developed and under-developed countries (Heaney, 1999). Hence, the secondary effects, refractoriness and poor response to available AEDs are major reasons to further

Abbreviations: MEAPR, methanol extract of *Anacyclus pyrethrum* root; AEAPR, aqueous extract of *Anacyclus pyrethrum* root; APR, *Anacyclus pyrethrum* root; SE, status epilepticus; WDS, wet-dog shakes; AEDs, antiepileptic drugs; PTZ, pentylenetetrazol; BCL, bicuculline; MES, maximal electroshock; ICES, increasing current electroshock; KA, kainic acid; DG, dentate gyrus

* Corresponding author.
E-mail address: bamhamed@uca.ma (S. Ba-M'hamed).

https://doi.org/10.1016/j.eplepsyres.2019.106225
Received 16 July 2019; Received in revised form 28 September 2019; Accepted 22 October 2019
Available online 24 October 2019
0920-1211/

research new treatment alternatives. In Morocco, healing with plants is considered a very commonly therapeutic alternative.

Anacyclus pyrethrum (L.) Link (Asteraceae) is mainly distributed in North Africa, Mediterranean region and India (Boulos, 1983). The plant is commonly known by Moroccan people as Tigandizt or AkkarKarha and traditionally is used for its antiepileptic and antirheumatic properties (Bellakhdar, 1997). In addition, this plant is reported to be used by the unani physicians to manage, control and treat various forms of epilepsy (Razi, 1997; Baitar, 1999). Several studies showed different activities of APR including antidiabetic (Satyanand et al., 2011), antioxidant (Sujith et al., 2011; Kalim et al., 2010) and anticancer (Mohammadi et al., 2017). These medicinal properties are due to the presence of polyphenols, tannins, coumarins, sterols, triterpenes, alkaloids and some trace metals (Hanane et al., 2014). The APR contains also polysaccharides (Bendjeddou et al., 2003) and *N*-isobutyl-dienediynamide (Boonen et al., 2012).

Beside local epidemiological local studies of Bellakhdar (1997) and Hmammouchi (1999) who reported some side effects as gastrointestinal disturbances and dermatological effects, no experimental study has shown side effects. However, in a sub-chronic study carried out by Sujith et al. (2012) on ethanolic extracts of Anacyclus pyrethrum root reported no effects on the normal growth of rats and no changes in hematological, biochemical or histopathological parameters. In addition, other authors report positive effects against long-term adverse outcomes, such as cancer (Mohammadi et al., 2017), liver and kidney damage (Usmani et al., 2016), and reproductive dysfunctions (Sharma et al., 2009). According to these published works, the APR had no treatment related toxicological abnormalities and can be considered as safe for long-term treatment.

Numerous models of epilepsy and epileptic seizures have been described **(**Loscher, 1999). However, not all these models of seizures and/or epilepsy can be used for all purposes, but researchers often do not discriminate between animal models of epilepsy and those of epileptic seizures although the difference may be important in interpretation of data obtained with such models, since, the clinical selection of an AED is based primarily on its efficiency for specific types of seizures and epilepsy (for review see Loscher, 2011),

In the line with these, many studies have demonstrated that extracts from APR possesses anticonvulsant potential in acute seizure models such as pentylenetetrazol, bicuculline, maximal electroshock and increasing current electroshock seizure **(**Pahuja et al., 2012; Zaidi et al., 2013**).** However, these simple screening seizure models are obviously not closely related to human epilepsy but represent models of epileptic seizures rather than models of epilepsy (Loscher, 1988; Bialer and White, 2010).

Kainic acid (KA) is the widely used model that mimics the clinical and neuropathological features of human temporal lobe epilepsy, the most common form of drug-refractory epilepsy (Nadler, 1981; Ben-Ari, 1985). It is an excitoxin, structural analogue of glutamate, which can bind to glutamate receptors and especially the kainate receptor (Hollmann and Heinemann, 1994). In fact, the systemic injection of KA induced status epilepticus (SE); which corresponds to prolonged and severe seizures, which, if not controlled, neuronal injury occurs (Ben-Ari, 1985; Ben-Ari and Cossart, 2000). In addition, the KA had a pro-inflammatory effect since it activates astrocytes and microglia in the brain (Zhang and Zhu, 2011).

So, from the foregoing, the anticonvulsive activity of APR was assessed in acute seizure models, but to our knowledge, not yet evaluated in animal models of epilepsy. In light of this, the present study was undertaken to investigate the anticonvulsive and protective effects of AEAPR and MEAPR in KA-induced-SE in mice. Here, to evaluate the neuroprotective effect of both extracts of APR, an immunohistochemical study was conducted in the hippocampus's mice by analyzing the expression of c-Fos and GFAP as a proxy of neuronal activation and neuro-inflammatory marker, respectively.

2. Materiel and methods

2.1. Animals

The animals used in this study are adult male Swiss mice (25–35 g) aged of 8 weeks and were obtained from the animal husbandry of the Faculty of Sciences of Marrakech, Morocco. The mice were singly housed and were handled daily throughout experiment period to minimize stress associated with social isolation (Manouze et al., 2019). The animals were kept under controlled environmental conditions (23 ± 1 °C; night-day cycle (12 h-12 h)) with *ad libitum* access to food (standard pellet diet) and water. The experimental procedures are carried out in accordance with the guidelines of European Council Directive: 2010/63/EU. Efforts were made to reduce the number of animals used and to minimize any animal suffering. Also, the study was approved by the Council Committee of Research Laboratories of the Faculty of Sciences of Marrakech.

2.2. Plant material

Anacyclus pyrethrum var. *pyrethrum (L.)* Link (Asteraceae) was collected in June 2014 from Marrakech region (Oukaïmeden, located at 2 600 m of altitude in the High-Atlas Mountains and 80 km from Marrakech). The identification of the plant was done in the department of Biology, Faculty of Sciences of Marrakech, by Professor A. Ouhammou. Sample of plant was deposited for reference at the Faculty's Herbarium (voucher number: Mark 8258).

2.3. Extracts preparation

The aqueous and methanolic extracts from APR used in this study were prepared as described in our previous study (Manouze et al., 2017). Briefly, fifty grams of the root powder was stirred with 1 l of distilled water for 24 h. The aqueous extract obtained was centrifuged (15 min, 1200 rpm) and the supernatant was lyophilized then stored at −20 °C until use. Four hundred grams of the powder of APR were extracted with methanol using Soxhlet apparatus. This methanolic extract was evaporated under vacuum and the residue was kept at −20 °C until further investigation. The yield of AEAPR and MEAPR was 20% and 21.8% w/w; respectively.

2.4. Quantitative phytochemical assessment

2.4.1. Determination of total phenolic content

The total phenolic content of AEAPR and MEAPR were determined by the Folin-Ciocalteu method described by Li et al. (2007). Briefly, a volume of 200 µl of each extract or standard was added to 1 ml of Folin-Ciocalteu reagent (10%). After 4 min, 400 µl of Na_2CO_3 (75 mg / ml) was added to the reaction medium. The mixture is allowed to react for 2 h at room temperature and then the absorbance was measured at 765 nm. Gallic acid is the standard used to establish the calibration curve from which the concentration of total phenols in the extracts was calculated. The result was expressed as mg of gallic acid equivalent per g of extract (mg GAE / g of extract).

2.4.2. Determination of total flavonoids contents

The flavonoid content of the extracts was determined by the aluminum trichloride method (Bahorun et al., 1996). One milliliter of the $AlCl_3$ solution (2% in methanol) was added to 1 ml of each extract or standard. After 10 min of incubation, the absorbance of the reaction mixture was measured at 430 nm. Total flavonoid was calculated by constructing a standard curve equation and expressed as mg of quercetin equivalent per g of extract (mg QE / g of extract).

2.4.3. Determination of total tannins contents

The total content of tannins was determined by the iron trichloride

($FeCl_3$) method (Heimler et al., 2006). A volume of 400 µl of each extract or standard was added to 3 ml of the vanillin solution (4% in methanol) and 1.5 ml of concentrated hydrochloric acid. After 15 min of incubation, the absorbance was measured at 500 nm. The concentration of tannins in the extracts was calculated according to the standard catechin graph. The results were expressed in microgram of mg equivalent per g of extract (mg CE / g of extract).

2.5. Pretreatment with Anacyclus pyrethrum extracts

The animals were randomly assigned to four groups (n = 6 per group): tap water control group, and tree KA treated groups corresponding to: KA group (receiving KA alone), AEAPR group (receiving AEAPR + KA) and MEAPR group (receiving MEAPR + KA). Prior the KA (Sigma-Aldrich, France) injection, the control and KA groups received tap water for 5 days, whereas treated groups received AEAPR or MEAPR diluted in tap water (5 g/l) for 5 supplementary days. KA and extract treated groups received an i.p. injection of KA at the dose of 30 mg/kg dissolved in distillated water at the 5th day of the experiment. The control group received a distillated water injection in the same day of KA administration. For each mouse (housed in individual cage), the water bottle was weighed for each day in a row at the same time of day to determine 24-h *ad libitum* fluid consumption. After injection of KA, animals were attended for 6 h to evaluate the latency for the onset of the first seizure episode and SE, as well as convulsive behavior according to Racine's scale (Racine, 1972). The number of wet-dog shakes (WDS), which is characteristic behavioral response (Ben-Ari, 1985), was counted for 6 h after i.p. injection of KA.

2.6. Immunohistochemistry

The effect of the APR extracts on c-FOS and GFAP expression in KA-induced SE was examined at 6 h after KA injection. The mice were anaesthetized with urethane 40% (1 g/kg, Sigma-Aldrich, France) and intracardially perfused with 0.9% saline followed by 4% paraformaldehyde (PFA). The brains were subsequently removed from the skull and post-fixed in 4% PFA for 24 h, then switched to a 30% sucrose solution for 24 h at 4 °C. Coronal sections of 40 µm were performed using a freezing microtome (Leica Microsystems, Germany). Slides containing the region of interest (hippocampus) were incubated 30 min with 1% H_2O_2 to inhibit endogenous peroxidase activity and rinsed extensively in phosphate buffered saline (PBS; pH 7.4) solution. They were pre-incubated with 1% bovine serum albumin (BSA; Serva electrophoresis GmbH, Germany) in PBST (PBS containing 0.3% Triton) for 60 min, with stirring and at room temperature. Then, the sections were incubated with the primary antibodies: anti-Fos produced in rabbit (Oncogène Science, France) or anti-GFAP produced in mice (Calbiochem, Germany) diluted at 1:3000 or 1:1000, respectively, in PBST-BSA for 24 h at 4 °C, at low speed shaking. After rinsing the sections with PBST, they are incubated, with secondary antibody biotinylated of c-FOS (anti-mouse IgG) and GFAP (anti-rabbit IgG), diluted 1:300 in PBST, overnight at 4 °C, followed by rinsing with PBS. The antigen-antibody binding is revealed by incubating the sections with the avidin-biotin complex kit (ABC, 1:200; vector laboratories, USA) for 2 h at room temperature with shaking, followed by rinsing with PBS. The revelation of the peroxidase activity is carried out in the dark with 0.025% of 3-3′-diaminobenzidine tetrahydrochloride (DAB) in Tris buffer (0.05 M; Prochilab, France) supplemented with hydrogen peroxide (0.006%). Processing was stopped by rinsing sections in PBS. The sections are collected and mounted on gelatine-coated slides, dried, dehydrated in increasing concentrations of alcohol and then cleared in xylene (5 min) and cover-slipped with Eukitt (Sigma-Aldrich,USA). The sections were examined under a light microscope for quantification of immunoreactive c-FOS and GFAP cells.

2.7. Quantitative analysis of c-FOS and GFAP

Photomicrographs of brain sections were taken at low (×25) and high magnification (×400) on Olympus BH-2 microscope (Olympus BH-2 U-PMTVC 4M25192, Japan) equipped with a digital camera (Olympus DP71). For data analysis, the densities of c-FOS or GFAP-positive cells were counted in regions CA1, CA3, and dentate gyrus of the dorsal hippocampus with ImageJ® software. Counting was done in five hippocampal slices from each animal and the results were averaged.

2.8. Statistical analysis

Statistical analysis was carried out in Sigma Plot 11.0 software. Body weight and water intake were analyzed by two-way ANOVA repeated measures followed by Tukey *post-hoc* analysis. To assess the animal survival rate, the Kaplan–Meier method was applied to mortality data, and comparisons of mortality data among groups were performed using the log-rank test. Moreover, seizure parameters were evaluated by one-way ANOVA followed by Tukey test. For immunohistochemical analyses, statistical comparisons were conducted by two-way ANOVA followed by Tukey *post-hoc* test. Statistical significance was considered when $p < 0.05$.

3. Results

3.1. Total phenolic, flavonoid and tannin content

According to the results reported in Table 1, it was found that total phenolic content of MEAPR and AEAPR were found to be 181.7 ± 1.5 and 192.1 ± 0.3 mg gallic acid / g equivalents/g plant extract, respectively. In addition, the total tannin detected in AEAPR and MEAPR was found to be 128.1 ± 0.2 and 100.5 ± 0.0 mg catechin equivalents / g plant extract, respectively. However, the flavonoid content was 19.2 ± 0.0 and 21.4 ± 0.1 mg quercetin equivalents / g plant extract in AEAPR and MEAPR. We noticed that there is no significant difference ($p > 0.05$) in the amounts of total phenolics, flavonoids and tannins between the two extracts.

3.2. Effects of AEAPR and MEAPR on water intake and body weight

In the present study, the MEAPR and AEAPR were administrated through drinking water in mice. We noticed that the water intake was increased in both MEAPR and AEAPR-treated mice. In contrast, control and KA groups had a constant consummation of water during the 5 days of experimental protocol (Fig. 1A). A two-way ANOVA repeated measures analysis was performed considering treatment and day as main factors. The results demonstrated a main effect of treatment ($F_{(3,15)} = 16.18$, $p < 0.001$) and day ($F_{(5,25)} = 5.65$, $p < 0.01$) on daily fluid consumption. Compared to control and KA groups, from the 3th day of experimental protocol, the treated groups consume significantly more MEAPR ($p < 0.01$ and $p < 0.05$; respectively) and AEAPR ($p < 0.01$ and $p < 0.05$; respectively) solutions. The daily dose taken by each mouse showed an average of 944.45 ± 3.96 or 919.67 ± 7.79 mg/kg/day of AEAPR or MEAPR respectively (Fig. 1B). The two way-ANOVA repeated measures analysis showed that dose

Table 1
Total phenolics, flavonoids and tannins contents of AEAPR and MEAPR.

	Total phenols (mg AGE/g)	*Total flavonoids* (mg QE/g)	Total tannins (mg CE/g)
AEAPR	192.1 ± 0.3	19.2 ± 0.0	128.1 ± 0.2
MEAPR	181.7 ± 1.5	21.4 ± 0.1	100.5 ± 0.0

Values expressed are means ± SEM of three parallel measurements.

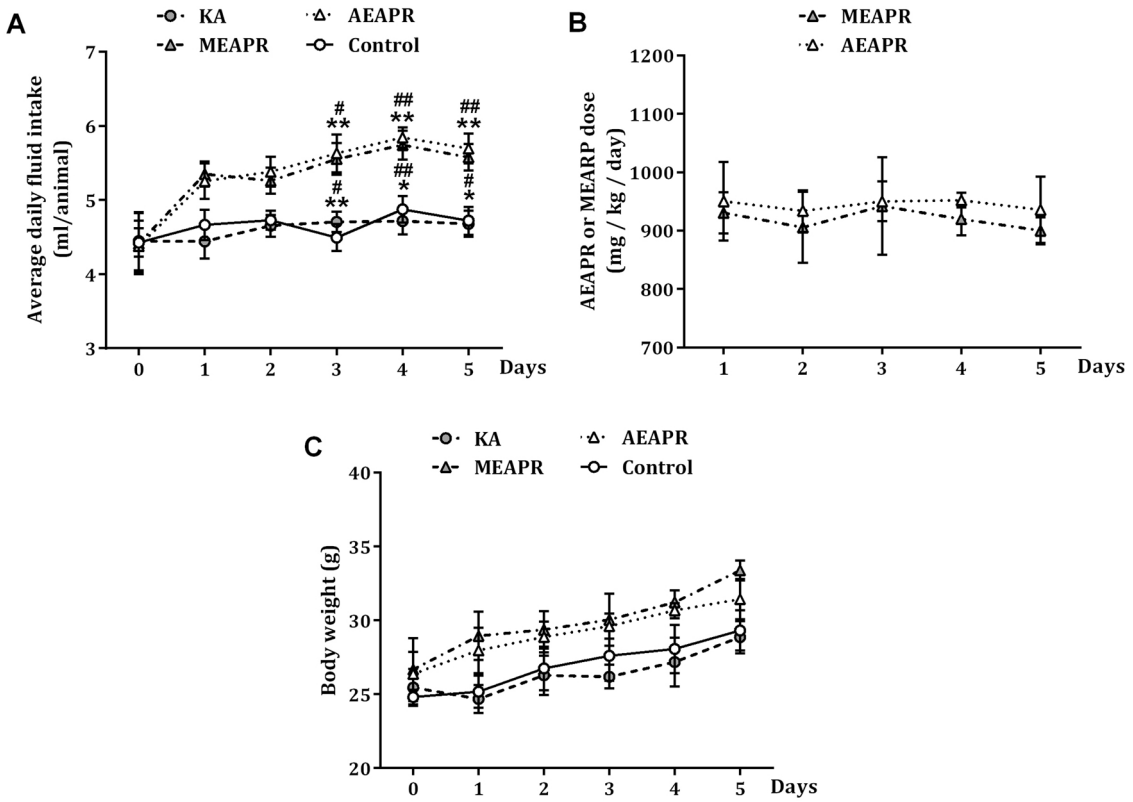

Fig. 1. Effects of pretreatment of AEAPR and MEAPR on water intake and body weight evolution in mice. **(A)** Average daily volume of water intake, **(B)** average daily concentration of AEAPR and MEAPR (mg/kg/day) and **(C)** body weight evolution of mice throughout the period of experiment. Data are presented as mean ± SEM of 6 mice/group, *p < 0.05 and **p < 0.01 refers to the comparison between the AEAPR- and MEAPR-treated groups *vs* KA group; #p < 0.05 and ##p < 0.01 refers to the comparison between the AEAPR- and MEAPR-treated groups *vs* control group.

intake of AEAPR or MEAPR (mg/kg/day) did not differ significantly between groups ($F_{(1, 10)} = 0.73$, $p > 0.05$) neither between days ($F_{(4, 10)} = 0.10$, $p > 0.05$). In addition, the pretreatment of both MEAPR and AEAPR did not affect the evolution of the body weight in mice (treatment: $F_{(3,15)} = 2.95$ and day: $F_{(5,25)} = 1.52$, $p > 0.05$; Fig. 1C).

3.3. Effect of pretreatment with AEAPR or MEAPR on KA-induced SE in mice

Our results showed that i.p injection of KA causes seizures in the all groups injected. However, pretreatment with MEAPR or AEAPR reduced the seizure parameters compared to the vehicle-pretreated group (Fig. 2). On the other hand, the KA injection in vehicle-pretreated group caused the death of one case, indicating a survival rate of 83.33%, whereas in the AEAPR and MEARP pretreated groups, no case of death was observed after KA injection (100%) (Fig. 2A). The log-rank test did not show any significant difference between KA group and treated groups ($x^2 = 3.00$,df = 3, p = 0.39). Data analysis by one-way ANOVA indicated a significant main effect of treatment on the latency of the first seizure ($F_{(2,15)} = 5.82$, $p < 0.05$; Fig. 2B), the seizure duration ($F_{(2,15)} = 7.47$, $p < 0.01$; Fig. 2C) and the number of WDS ($F_{(2,15)} = 11.76$, $p = 0.01$; Fig. 2D). In fact, Tukey *post-hoc* test confirmed that the pretreatment with MEAPR ($p < 0.05$) and AEPAR ($p < 0.05$) delayed significantly the onset of seizures compared to the vehicle. In addition, the seizure duration and the number of WDS was deceased significantly in the mice pretreated with MEAPR ($p < 0.01$ and $p < 0.05$; respectively) or AEAPR ($p < 0.05$ and $p < 0.01$; respectively) in comparison with the vehicle-pretreated group.

Moreover, one-way ANOVA analysis was performed considering treatment as main factor. The results revealed a significant effect between different groups in the severity of seizure ($F_{(2,15)} = 9.73$, $p < 0.01$; Fig. 2E) and the latency of SE ($F_{(2,15)} = 6.72$, $p < 0.05$; Fig. 2F). As compared to the vehicle-pretreated group, MEAPR and AEAPR-pretreated groups showed significantly less severe seizures ($p < 0.01$ and $p < 0.01$; respectively). Additionally, our data indicated that both AEAEP and MEAPR pretreatments delayed significantly ($p < 0.05$) the appearance of SE as compared to the vehicle pretreated group. We noticed that no significant difference was shown between AEAPR and MEAPR-treated groups in all calculated seizure parameters.

3.4. Effects of AEAPR and MEAPR on c-FOS and GFAP expression in hippocampus of KA-induced SE in mice

We evaluated the activation of hippocampus areas following 6 h of KA post-injection by c-FOS marker. We observed a high density of Fos immunoreactive neurons of hippocampus in all KA-treated mice. This increase in c-FOS expression was seen in the DG, CA1 and CA3 areas (Fig. 3A).Two-way ANOVA showed a main effect of treatment ($F_{(3,48)} = 111.4$, $p < 0.001$) and a significant effect of the hippocampus area's ($F_{(2,48)} = 10.01$, $p < 0.001$; Fig. 3B); whereas, the interaction treatment x hippocampus area's had no effect ($F_{(6,48)} = 0.76$, $p > 0.05$). Indeed, *post-hoc* test showed that FOS-immunoreactivity of the KA group was significantly higher in the hippocampus regions as compared to the control group ($p < 0.001$). In comparison to the KA group, the induction of FOS-immunoreactivity was significantly fewer in the CA1 ($p < 0.001$), CA3 ($p < 0.001$) and DG ($p < 0.001$) of AEAPR and MEARP-treated mice. We observed that the number of FOS-immunoreactivity found in the CA1/CA3 or DG was not different between AEAPR-and MEAPR-treated groups ($p > 0.05$).

To examine whether AEAPR and MEAPR countered the inflammatory processes in KA-injected mice, the activation of astrocytes

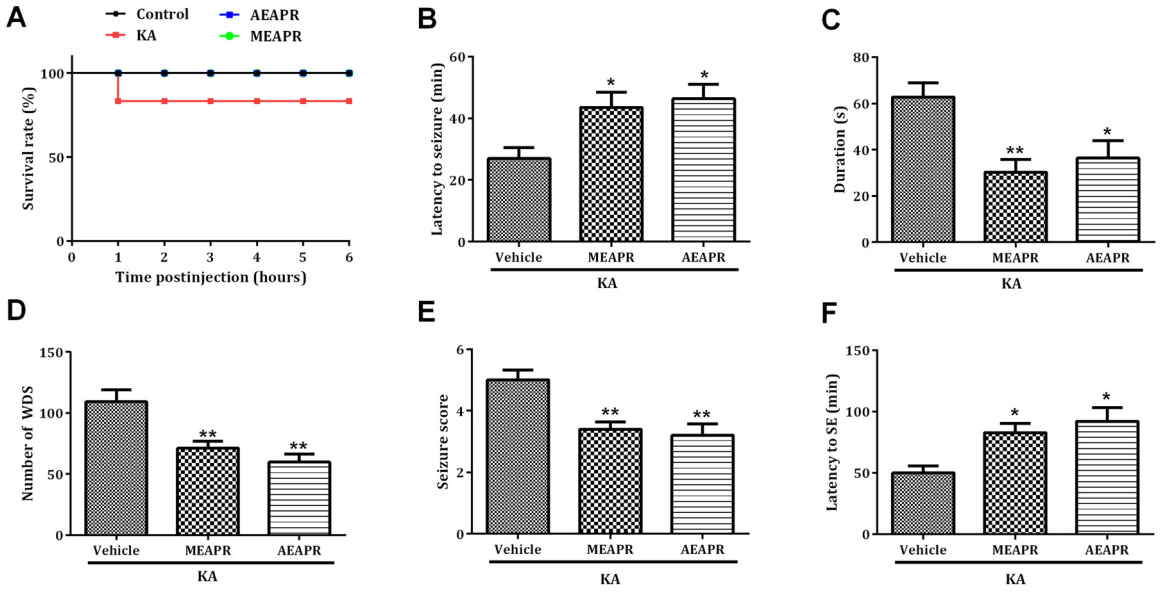

Fig. 2. Effect of pretreatment with AEAPR or MEAPR on KA-induced SE in mice.
(A) Kaplan-Meier's presentation of the survival rate after treatments, **(B)** latency to seizure, **(C)** seizure duration,**(D)** number of WDS, **(E)** seizure score and **(F)** latency to SE was shown for AEAPR or MEAPR-treated groups and vehicle-treated group. Data are expressed as mean ± SEM. *p < 0.05 and **p < 0.01 compared with KA group.

after administering KA was analyzed by detecting the expression of GFAP, a neuro-inflammatory marker. The results of GFAP immunohistochemistry analysis revealed a net increase in positive neurons in KA-treated mice in all regions of hippocampus 6 h after KA injection (Fig. 4A). Two-way ANOVA demonstrated a significant effect of treatment ($F_{(3,48)} = 79.25$, $p < 0.001$), a significant effect of hippocampus region ($F_{(2,48)} = 8.89$, $p < 0.001$) and the interaction of treatment x hippocampus region ($F_{(6,48)} = 0.38$, $p > 0.05$) had no effect on the GFAP expression (Fig. 4B). The *post-hoc* analysis showed a significant increase of GFAP expression in the CA1, CA3 and DG in the KA group ($p < 0.001$) as compared to control group; whereas, both of AEAPR and MEAPR groups did not differ of control group ($p > 0.05$). However, a significant decrease in the number of labeled neurons was observed in the hippocampus (CA1, CA3, and DG) of AEAPR and MEAPR groups as compared to KA group ($p < 0.001$).

4. Discussion

Our study demonstrated the protective role of AEAPR and MEAPR against seizure and SE induced by KA injection in mice. Also, our findings illustrated the beneficial role of both AEAPR and MEAPR in attenuating neuronal activity and neuroinflammation. First, we given both of AEAPR and MEAPR to mice *via* drinking water to reduce the stress related to drug administration. In fact, conventional route for substance administration in rodents almost involve restraining the animal (Deacon, 2006); which leads to increase the stress level that can influence parameters under study. In fact, our results showed that water intake was significantly increased in AEAPR and MEAPR-treated groups compared to vehicle-treated group. It is well known that rodents naturally have a preference for sweet solutions. Furthermore, *Anacyclus pyrethrum* contains many secondary metabolites such as reducing sugar and sugar (Subasri and Ahmed John, 2016). Therefore, the increase of water intake is due to the sweetness of both AEAPR and MEAPR. Moreover, body weight, a parameter commonly analyzed in toxicology studies, was not affected by the administration of AEAPR and MEAPR in mice. This result in accord with our previous study (Manouze et al., 2017), where we reported that both AEAPR and MEARP up to the dose of 5000 mg/kg are non-toxic and had no effect on the body weight of mice. Similarly, Sujith et al. (2012) have shown in rats that sub-chronic administration of ethanolic extract of APR at dose of 1 000 mg/kg had no effect on the normal growth. Taken together, Anacyclus pyrethrum root could testify to its safety and could stand as assurance for medicinal its use for long-term treatment.

Then, we screened MEAPR and AEAPR for its anticonvulsive and protective effect against seizures and SE induced by KA injection. We used KA-induced SE model because of its behavioral and pathological similarities to the human temporal lobe epilepsy, the most common type of epilepsy (Nadler, 1981). The systemic administration of KA to animals resulted in limbic SE, followed days or weeks later by the development of spontaneous recurrent seizures (Ben-Ari and Cossart, 2000). Typically, the KA model develops wet-dog shakes as a characteristic behavioral response (Ben-Ari, 1985). In the present study, KA injected intraperitoneally at dose of 30 mg/kg produced behavioral changes as well as convulsions in all animals; with minimal mortality. This finding is consistent with the results obtained when other research group applied the same dose of KA (Ahmad et al., 2013); where they confirmed that bolus dose of KA 30 mg/kg is sufficient to induce significant SE characteristics and minimal mortality.

However, the administration of AEAPR at dose of 944.45 mg/kg/day or MEAPR at dose of 919.67 mg/kg/day before a KA injection resulted in a protective effect against seizures; as revealed by significant increase in the latencies to onset of seizure; whereas, the duration and severity of seizures were reduced significantly as compared to the vehicle-treated mice. Comparatively to Pahuja et al. (2012) study, the hydroalcoholic extract from APR at dose of 1000 mg/kg produced only 50% protection against generalized tonic clonic seizures in PTZ model; whereas, at dose of 500 mg/kg, a complete protection was observed against tonic hind limb extension in MES model. Taken together, the effectiveness of APR in controlling seizures depends of the type of seizures or epilepsy. Moreover, the origin of plant and the extraction method used may play role in the variation of the content of secondary metabolite (Thouri et al., 2017). On the other hand, our data showed that the number of WDS was decreased and the latency of SE was increased (77.9 and 91.9%, respectively) by the pretreatment with AEAPR and MEAPR in KA-induced-SE model. Given that this model representsthe refractoryepilepsy to medication (Nadler, 1981), the anticonvulsive effect of AEAPR and MEAPR in KA-induced-SE model seems to be more relevant than it is in the acute seizure models (MES

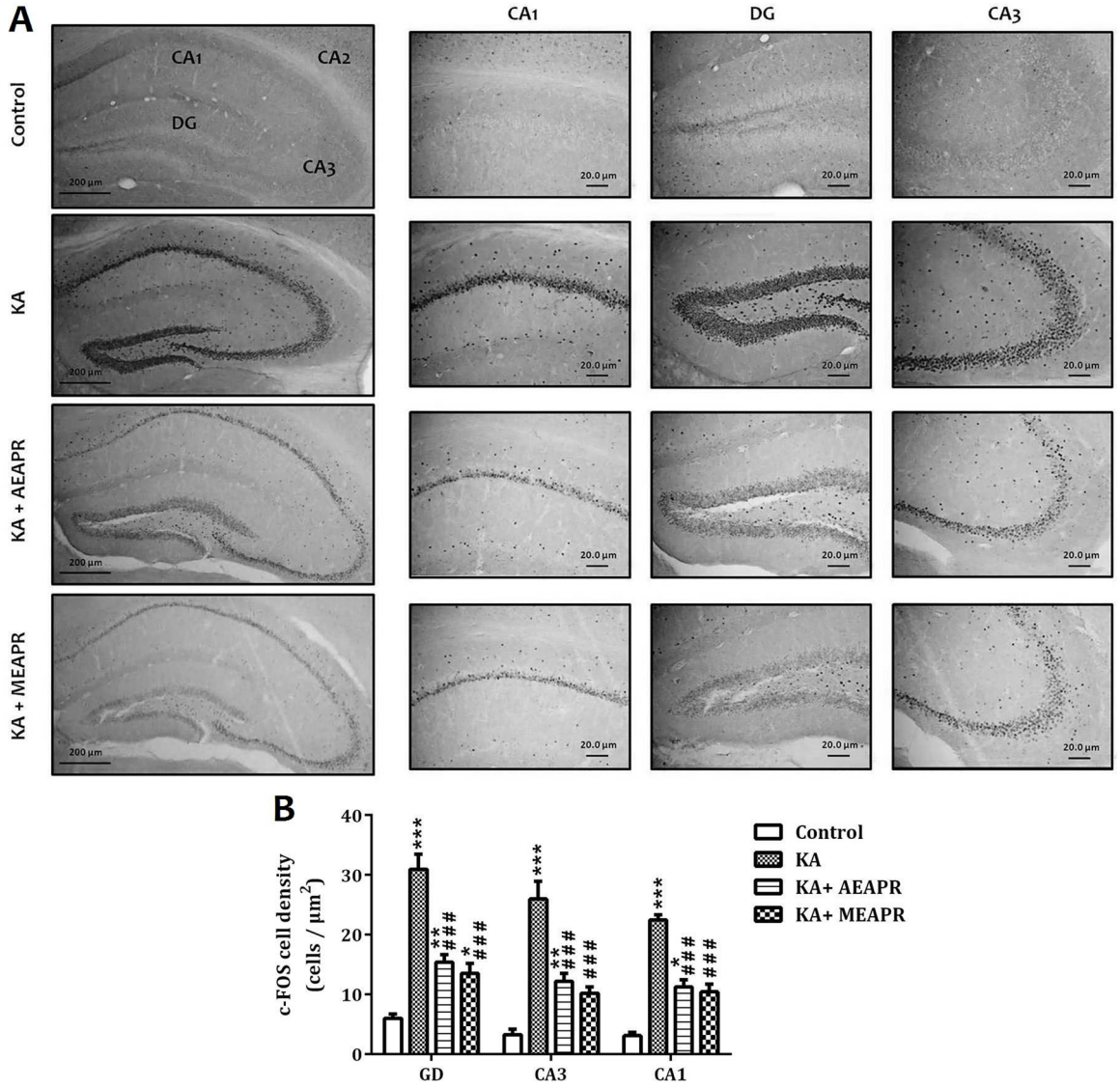

Fig. 3. Effect of AEAPR and MEAPR pretreatment on c-FOS expression in mice hippocampus. **(A)** Photomicrographs showing c-FOS labelled nuclei in hippocampus using immunohistochemistry 6 h after the KA injection. **(B)** Quantification of c-FOs expression in the hippocampus from differently treated groups. *p < 0.05, **p < 0.01, ***p < 0.001 compared to control group; ###p < 0.001 compared to KA group.

and PTZ).

It is proposed that KA act, in part, by excitotoxicity process. The activation of AMPA and kainate receptors by KA leads to an increase of glutamate release through presynaptic receptors (Farooqui et al., 2008). By cons, the high level of glutamate allows calcium ions (Ca^{2+}) to enter the cell (Sairazi et al., 2015). High level of intracellular calcium activates various pathways to generate free radicals (Hsieh et al., 1999). Hence, the anticonvulsant effect of the *Anacyclus pyrethrum* plant could be explained by the inhibition of the binding between KA and glutamate receptors. It is likely that *Anacyclus pyrethrum* may contain constituents that may be involved in this action.

Effectively, the results obtained indicated a relatively high level of total phenolic (181.7 and 192.1 mg AGE / g, respectively) and tannins (100.5 and 128.1 mg CE / g, respectively) in MEAPR and AEAPR. However, the flavonoid content was 19.2 mg QE / g in AEAPR and 21.4 mg QE / g in MEAPR. Thus confirming what has been assumed regarding its content of phenolic compounds in the preliminary screening (Manouze et al., 2017). Comparatively, lower total phenolic content, was reported in the same species from india (Sujith et al. (2011). In addition, higher total flavonoid content was indicated in the same species from Algeria (Selles et al., 2012). The difference in amounts of phenols is probably related to geographical, environmental, genetic factors (Papoulias et al., 2009), as well as the degree of maturation of the plant (Aganga and Mosase, 2001), which may play role in such a large variation.

There is an increasing evidence for the pharmacological effects of polyphenols, including neuroprotective and antiepileptic properties (Lasoń and Leśkiewicz, 2013; Kwon et al., 2019). For example, the chlorogenic acid as polyphenolic compound was presented in *Anacyclus pyrethrum* (Kalim et al., 2010), exerted antiepileptic and neuroprotective activity by suppressing glutamate receptors (Aseervatham et al., 2016).

Growing evidence indicates that KA increases ROS production (Dugan et al., 1995; Hsieh et al., 1999). However, antioxidants would serve as agents that can inhibit the production of free radicals (Frantseva et al., 2000). Several studies demonstrated the antioxidant potential of *Anacyclus pyrethrumin vitro* (Kalim et al., 2010; Selles et al., 2012) and *in vivo* (Pahuja et al., 2012). Indeed, the previous study indicated that AEAPR and MEAPR have free radical scavenging effect and antioxidant activity *in vitro* (Manouze et al., 2017). Thus, the anticonvulsive activity of MEAPR and AEAPR can attributed to its antioxidant effect.

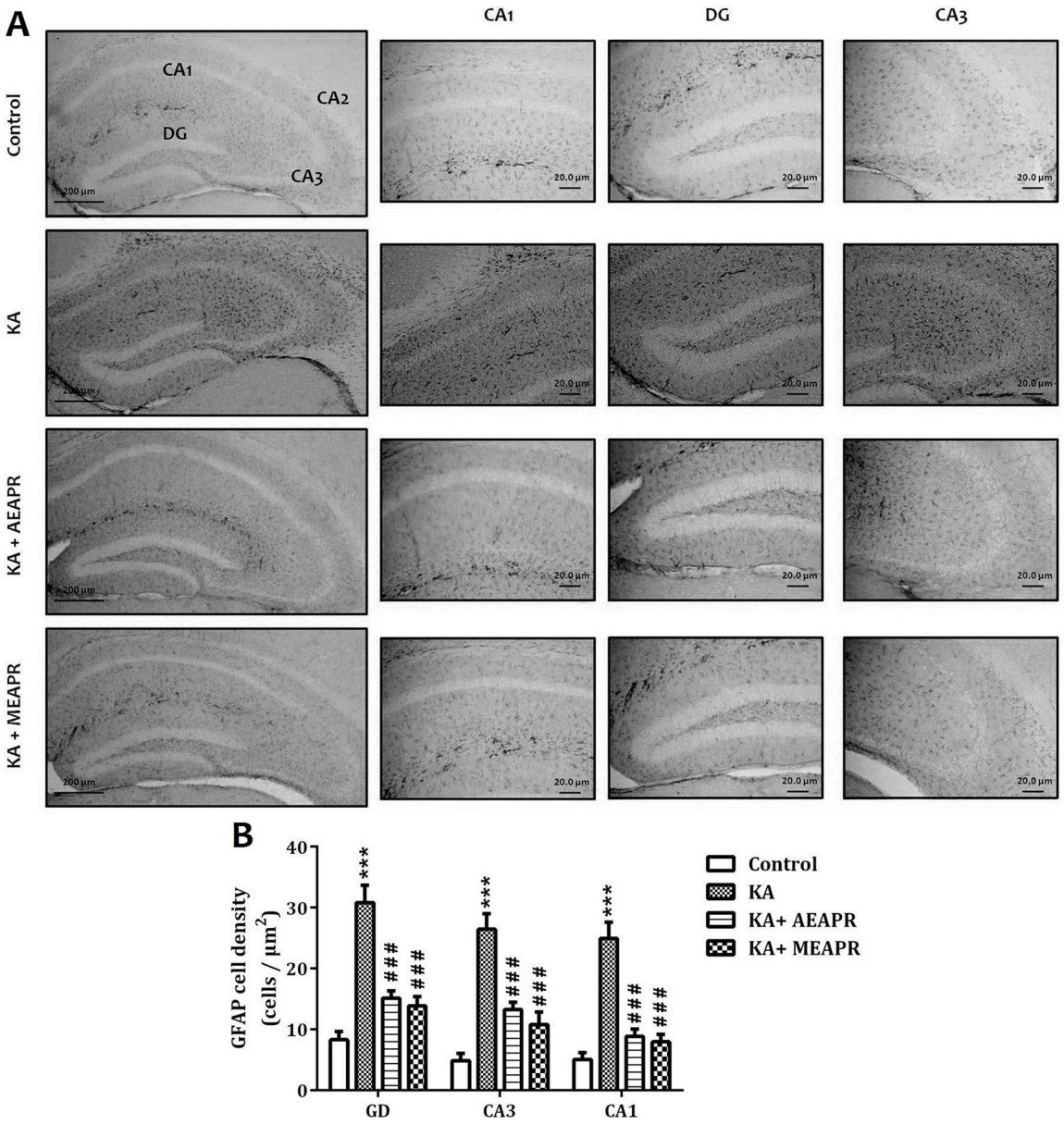

Fig. 4. Effect of AEAPR and MEAPR pretreatment on GFAP expression in mice hippocampus. **(A)** Photomicrographs showing the GFAP immunoreactive cells in the hippocampus. **(B)** Quantification of GFAP expression in the hippocampus from differently treated groups. ***$p < 0.001$ compared to control group; $^{###}p < 0.001$ compared to KA group.

Next, we studied neural activity in the hippocampus of mice, a first target brain region after systemic injection of KA (Tanaka et al., 1992), using c-FOS marker. Our data indicated that c-FOS, transcription factor of the immediate-early gene family (Herdegen and Leah, 1998), was mainly expressed in different subfields of the hippocampus (CA1, CA3 and GD) after 6 h of KA injection. There is evidence demonstrating that KA increases the binding of AP-1 (family member proteins of c-FOS) to DNA in two phases: the first phase (2–6 h) is related with seizures and the second phase (48–72 h) is associated to the period of delayed neuronal cell death (Kaminska et al., 1994). It has been reported that the increases of c-FOS immunoreactivity in the CA1, CA3 and DG correspond to neuronal activity during SE (Yoo et al., 1996). Moreover, our findings are consistent with several studies demonstrating that c-FOS expression was highly increased in the hippocampus following KA treatment. Also, it has been showed that c-FOS is a sensory marker with excitotoxicity induced by KA (Shin et al., 2009; Lin et al., 2013). Otherwise, our data showed that the pretreatment with MEAPR and AEAPR reduced significantly the expression of c-FOS in the CA1, CA3 and DG of hippocampus. There is a correlation between behavioral manifestations and hippocampal neuronal activity; which suggest that AEAPR and MEAPR induced protection against KA-induced seizures may be to their ability to reduce the expression of c-FOS protein.

In the other hand, a number of studies have indicated that inflammation might be a consequence and also a cause of epilepsy (Choi et al., 2009; Vezzani et al., 2011). It has also been described a pro-inflammatory effect of KA, since it activates astrocytes and microglia in the brain (Zhang and Zhu, 2011). Therefore, we investigated the influence of AEAPR and AEAPR on the neuroinflammation by GFAP marker in KA-induced seizures. This is how we observed an increase expression of GFAP, a neuro-inflammatory marker assessing the reactive state of astrocytes, in response to KA injection. This finding is in line with previous studies showing that a systemic injection of KA resulted in large increase of microglia activation (Zheng et al., 2011; Lin et al., 2015). However, it was markedly reduced by the pretreatment with AEAPR and MEAPR in the hippocampus (CA1, CA3 and DG). Thus, *Anacyclus pyrethrum* extracts may also exert protective effect against

seizures in KA-treated mice through its anti-inflammatory activity. In fact, we have showed previously that AEAPR and MEAPR have anti-inflammatory activity (Manouze et al., 2017). In addition, alkamides constituents of *Anacyclus pyrethrum* inhibited the action of 5-lipoxygenase (LOX) and cyclooxygenase (COX) enzymes (Rimbau et al., 1999). Thus, speculating that the neuroprotective effect of AEAPR and MEAPR in KA animal model is at least partially attributable to their anti-inflammatory effect is reasonable.

5. Conclusion

Our study showed that AEAPR and MEAPR generate anticonvulsive activity in mice. In addition, we showed that both of AEAPR and MEAPR were effective in attenuating the neuronal activation and neuroinflammation caused by KA injection. The plausible reason for this would be attributed to the anti-inflammatory and antioxidant activity of AEAPR and MEAPR. These findings enhance the understanding of the actions of AEAPR and MEAPR in the brain and suggest that this natural compound may be useful to produce neuronal protection in epilepsy and other brain disorders that are related with excitotoxicity and neuroinflammation. Yet, further investigations must be conducted to isolate the active compounds responsible for the anticonvulsant effect of APR.

Declaration of Competing Interest

All authors declare that the study was done in absence of any conflict of interest.

Acknowledgments

This work was supported by NEUREN Project (PIRSES-GA-2012-318997). We thank A. REGRAGUI for his support by providing us experimental animals.

References

Aganga, A.A., Mosase, K.W., 2001. Tannins content, nutritive value and dry matter digestibility of Lonchocarous capussa, Ziziphus mucropata, Sclerocarya birrea, Kirkia acuminata and Rhus lancea seeds. Anim. Feed Sci. Technol. 91 (1–2), 107–113.

Ahmad, N., Yow, H., Makmor-Bakry, M., 2013. Seizure attainment and mortality of mice in kainate-induced status epilepticus. Int. J. Pharm. Pharm. Sci. 5, 145–147.

Arroyo, S., Brodie, M.J., Avanzini, G., Baumgartner, C., Chiron, C., Dulac, O., French, J.A., Serratosa, J.M., 2002. Is refractory epilepsy preventable? Epilepsia 43, 437–444. https://doi.org/10.1046/j.1528-1157.2002.38501.x.

Aseervatham, G.S.B., Suryakala, U., Sundaram, S., Bose, P.C., Sivasudha, T., 2016. Expression pattern of NMDA receptors reveals antiepileptic potential of apigenin 8-C-glucoside and chlorogenic acid in pilocarpine induced epileptic mice. Biomed. Pharmacother. 82, 54–64. https://doi.org/10.1016/j.biopha.2016.04.066.

Baitar, I., 1999. Aljami Li Mufradat Al Advia Wal Aghziya (Urdu translation). CCRUM publication, New Delhi.

Bahorun, T., Gressier, B., Trotin, F., Brunete, C., Dine, T., Vasseur, J., Gazin, J.C., Pinkas, M., Luycky, M., Gazin, M., 1996. Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. Arzneim. Forsch. 46, 1086–1089.

Bellakhdar, J., 1997. La Pharmacopée Marocaine Traditionnelle: Médecine arabe ancienne et savoirs populaires. Ibis Press, Paris.

Ben-Ari, Y., 1985. Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. Neuroscience 14, 375–403. https://doi.org/10.1016/0306-4522(85)90299-4.

Ben-Ari, Y., Cossart, R., 2000. Kainate, a double agent that generates seizures: two decades of progress. Trends Neurosci. 23, 580–587. https://doi.org/10.1016/S01662236(00)01659-3.

Bendjeddou, D., Lalaoui, K., Satta, D., 2003. Immunostimulating activity of the hot water soluble polysaccharide extracts of *Anacyclus pyrethrum*, *Alpinia galanga* and *Citrullus colocynthis*. J. Ethnopharmacol. 88, 155–160. https://doi.org/10.1016/S0378-8741(03)00226-5.

Bialer, M., White, H.S., 2010. Key factors in the discovery and development of new antiepileptic drugs. Nat. Rev. Drug Discov. 9, 68–82.

Boonen, J., Sharma, V., Dixit, V.K., Burvenich, C., De Spiegeleer, B., 2012. LC-MS N-alkylamide profiling of an ethanolic *Anacyclus pyrethrum* root extract. Planta Med. 78, 1787–1795. https://doi.org/10.1055/s-0032-1315371.

Boulos, L., 1983. Medicinal Plants of North Africa. Reference Publications Inc, Michigan.

Choi, J., Nordli, D.R., Alden, T.D., DiPatri, A., Laux, L., Kelley, K., Rosenow, J., Schuele, S.U., Rajaram, V., Koh, S., 2009. Cellular injury and neuroinflammation in children with chronic intractable epilepsy. J. Neuroinflammation 6, 38. https://doi.org/10.1186/1742-2094-6-38.

Deacon, R.M., 2006. Housing, husbandry and handling of rodents for behavioral experiments. Nat. Protoc. 1, 936–946. https://doi.org/10.1038/nprot.2006.120.

Devinsky, O., Vickrey, B.G., Cramer, J., Perrine, K., Hermann, B., Meador, K., Hays, R.D., 1995. Development of the quality of life in epilepsy inventory. Epilepsia 36, 1089–1104. https://doi.org/10.1111/j.1528-1157.1995.tb00467.x.

Dugan, L.L., Sensi, S.L., Canzoniero, L.M., Handran, S.D., Rothman, S.M., Lin, T.S., Goldberg, M.P., Choi, D.W., 1995. Mitochondrial production of reactive oxygen species in cortical neurons following exposure to N-methyl-D-aspartate. J. Neurosci. 15, 6377–6388. https://doi.org/10.1523/JNEUROSCI.15-10-06377.1995.

Farooqui, A.A., Ong, W.Y., Lu, X.R., Halliwell, B., Horrocks, L.A., 2008. Neurochemical consequences of kainate-induced toxicity in brain: involvement of arachidonic acid release and prevention of toxicity by phospholipase A (2) inhibitors. Brain Res. Rev. 38, 61–78. https://doi.org/10.1016/S0169-328X(01)00214-5.

Frantseva, M.V., Velazquez, J.L., Hwang, P.A., Carlen, P.L., 2000. Free radical production correlates with cell death in an in vitro model of epilepsy. Eur. J. Neurosci. 12, 1431–1439. https://doi.org/10.1046/j.1460-9568.2000.00016.x.

Hanane, E., Aminata, S., Fatima, E., Amar, B., Mohamed, A.E., Touriya, Z., 2014. Phytochemical study of *Anacyclus pyrethrum* (L.) of Middle Atlas (Morocco), and in vitro study of antibacterial activity of pyrethrum. Adv. Nat. Appl. Sci. 8, 131–140.

Heaney, D., 1999. The pharmacoeconomics of the new antiepileptic drugs. Epilepsia 40, 25–31. https://doi.org/10.1111/j.1528-1157.1999.tb00944.x.

Herdegen, T., Leah, J.D., 1998. Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Res. Rev. 28, 370–490. https://doi.org/10.1016/S0165-0173(98)00018-6.

Heimler, D., Vignolini, P., Dini, M.G., Vincieri, F.F., Romani, A., 2006. Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. Food. Chem. 99, 464–469.

Hmammouchi, Mohamed, 1999. Les plantes médicinales et aromatiques marocaines. Edition Imprimeries de Fedala, Rabat, Maroc 389 p.

Hollmann, M., Heinemann, S., 1994. Cloned glutamate receptors. Annu. Rev. Neurosci. 17, 31–108. https://doi.org/10.1146/annurev.ne.17.030194.000335.

Hsieh, C.L., Chiang, S.Y., Tang, N.Y., Hsieh, C.T., 1999. Time course of behavior and generation of free radicals in kainic acid-induced epileptic seizures. Acta Neurol. Taiwan 8, 63–64.

Kalim, M.D., Bhattacharyya, D., Banerjee, A., Chattopadhyay, S., 2010. Oxidative DNA damage preventive activity and antioxidant potential of plants used in Unani system of medicine. BMC Complement. Altern. Med. 10, 77. https://doi.org/10.1186/1472-6882-10-77.

Kaminska, B., Filipkowski, R.K., Zurkowska, G., Lason, W., Przewlocki, R., Kaczmarek, L., 1994. Dynamic changes in the composition of the AP-1 transcription factor DNA-binding activity in rat brain following kainate-induced seizures and cell death. Eur. J. Neurosci. 6, 1558–1566. https://doi.org/10.1111/j.1460-9568.1994.tb00546.x.

Kwan, P., Schachter, S.C., Brodie, M.J., 2011. Drug-resistant epilepsy. N. Engl. J. Med. 365, 919–926. https://doi.org/10.1056/NEJMra1004418.

Kwon, J.Y., Jeon, M.T., Jung, U.J., Kim, D.W., Moon, G.J., Kim, S.R., 2019. Perspective: therapeutic potential of flavonoids as alternative medicines in epilepsy. Adv. Nutr. 10, 778–790.

Lasoń, W., Leśkiewicz, M., 2013. Effect of plant polyphenols on seizures – animal studies. J. Epileptol. 21 (2). https://doi.org/10.1515/joepi-2015-0007. ISSN (Online) 2300-0147.

Li, N., Wu, J.L., Hasegawa, T., Sakai, J., Bai, L.M., Wang, L.Y., Kakuta, S., Furuya, Y., Ogura, H., Kataoka, T., Tomida, A., Tsuruo, T., Ando, M., 2007. Bioactive polyketides from Peperomia duclouxii. J. Nat. Prod. 70 (6), 998–1001.

Lin, K.C., Wang, C.C., Wang, S.J., 2013. Bupropion attenuates kainic acid-induced seizures and neuronal cell death in rat hippocampus. Prog. Neuropsychopharmacol. Biol. Psychiatry 45, 207–214. https://doi.org/10.1016/j.pnpbp.2013.05.016.

Lin, T.Y., Lu, C.W., Wang, S.J., Huang, S.K., 2015. Protective effect of hispidulin on kainic acid-induced seizures and neurotoxicity in rats. Eur. J. Pharmacol. 755, 6–15. https://doi.org/10.1016/j.ejphar.2015.02.041.

Loscher, W., 2011. Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. Seizure 20, 359–368.

Loscher, W., 1999. Animal models of epilepsy and epileptic seizures. In: Eadie, M.J., Vajda, F. (Eds.), Antiepileptic Drugs. Handbook of Experimental Pharmacology. Springer, Berlin, pp. 19–62.

Loscher, W., 1988. Schmidt D. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Res. 2, 145–181.

Manouze, H., Bouchatta, O., Gadhi, A.C., Bennis, M., Sokar, Z., Ba-M'hamed, S., 2017. Anti-inflammatory, antinociceptive, and antioxidant activities of methanol and aqueous extracts of *Anacyclus pyrethrum* roots. Front. Pharmacol. 8, 598. https://doi.org/10.3389/fphar.2017.00598*.

Manouze, H., Ghestem, A., Poillerat, P., Bennis, M., Ba-M'hamed, S., Jean-Jacques Benoliel, J.J., Becker, C., Bernard, C., 2019. Effects of single cage housing on stress, cognitive and seizure parameters in the rat and mouse pilocarpine models of epilepsy. eNeuro. https://doi.org/10.1523/ENEURO.0179-18.2019.

Mohammadi, A., Mansoori, B., Baradaran, P.C., Baradaran, S.C., Baradaran, B., 2017. *Anacyclus pyrethrum* extract exerts anticancer activities on the human colorectal cancer cell line (hct) by targeting apoptosis, metastasis and cell cycle arrest. J. Gastrointest. Cancer 48, 333–340. https://doi.org/10.1007/s12029-016-9884-7.

Nadler, J.V., 1981. Kainic acid as a tool for the study of temporal lobe epilepsy. Life Sci. 29, 2031–2042. https://doi.org/10.1016/0024-3205(81)90659-7.

Pahuja, M., Mehla, J., Reeta, K.H., Joshi, S., Gupta, Y.K., 2012. Root extract of *Anacyclus pyrethrum* ameliorates seizures, seizure-induced oxidative stress and cognitive impairment in experimental animals. Epilepsy Res. 98, 157–165. https://doi.org/10.1016/j.eplepsyres.2011.09.006.

Papoulias, E., Siomos, A., Koukounaras, A., Gerasopoulo, s D., Kazakis, E., 2009. Effects of genetic, pre-and post-harvest factors on phenolic content and antioxidant capacity of white asparagus spears. Int. J. Mol. Sci. 10 (12), 5370–5380.

Racine, R.J., 1972. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr. Clin. Neurophysiol. 32, 281–294. https://doi.org/10.1016/0013-4694(72)90177-0.

Razi, Z., 1997. Al Hawi Fi Tib (Urdu translation). CCRUM, New Delhi.

Rimbau, V., Cerdan, C., Vila, R., Iglesias, J., 1999. Antiinflammatory activity of some extracts from plants used in the traditional medicine of North-African countries (II). Phytother. Res. 13, 128–132. https://doi.org/10.1002/(SICI)1099-1573(199903)13:2<128::AID-PTR399>3.0.CO;2-7.

Sairazi, M., Shafika, N., Sirajudeen, K.N.S., Asari, M.A., Muzaimi, M., Mummedy, S., Sulaiman, S.A., 2015. Kainic acid-induced excitotoxicity experimental model: protective merits of natural products and plant extracts. Evid. Complement. Alternat. Med. 2015, 972623. https://doi.org/10.1155/2015/972623.

Satyanand, T.M., Mansoori, H., Singh, N.K., Shivhare, M.K., Bhardwaj, P., Singh, R.K., 2011. Antidiabetic effect of *Anacyclus pyrethrum* DC in alloxan induced diabetic rats. Eur. J. Biol. Sci. 3, 117–120.

Selles, C.H., El amine, D.M., Allali, H., Tabti, B., 2012. Evaluation of antimicrobial and antioxidant activities of solvent extracts of *Anacyclus pyrethrum* L., from Algeria. Mediterr. J. Chem. 2, 408–415. https://doi.org/10.13171/mjc.2.2.2012.01.11.22.

Sharma, V., Thakur, M., Chauhan, N.S., Dixit, V.K., 2009. Evaluation of the anabolic, aphrodisiac & reproductive activity of Anacyclus pyrethrum DC in male rats. Sci. Pharm. 77, 97–110.

Shin, E.J., Jeong, J.H., Kim, A.Y., Koh, Y.H., Nah, S.Y., Kim, W.K., Ko, K.H., Kim, H.J., Wie, M.B., Kwon, Y.S., Yoneda, Y., Kim, H.C., 2009. Protection against kainate neurotoxicity by ginsenosides: attenuation of convulsive behavior, mitochondrial dysfunction, and oxidative stress. J. Neurosci. Res. 87, 710–722. https://doi.org/10.1002/jnr.21880.

Subasri, G., Ahmed John, S., 2016. Screening of phytochemical compounds, trace metals and antimicrobial activity of *Anacyclus pyrethrum*. Int. J. Adv. Sci. Res. 2, 032–037. https://doi.org/10.7439/ijasr.v2i1.2891.

Sujith, K., Darwin, C.R., Suba, V., 2011. Antioxidant activity of ethanolic root extract of Anacyclus pyrethrum. Int. Res. J. Pharm. Pharmacol. 2, 222–226.

Sujith, K., Darwin, R., Suba, V., 2012. Toxicological evaluation of ethanolic extract of *Anacyclus pyrethrum* in albino Wistar rats. Asian Pac. J. Trop. Dis. 437, 441. https://doi.org/10.1016/S2222-1808(12)60096-6.

Tanaka, T., Tanaka, S., Fujita, T., Takano, K., Fukuda, H., Sako, K., Yonemasu, Y., 1992. Experimental complex partial seizures induced by a microinjection of kainic acid into limbic structures. Prog. Neurobiol. 38, 317–334. https://doi.org/10.1016/0301-0082(92)90023-8.

Thouri, A., Chahdoura, H., El Arem, A., Omri Hichri, A., Ben Hassin, R., Achour, L., 2017. Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). BMC Complement. Altern. Med. 17 (May (1)), 248. https://doi.org/10.1186/s12906-017-1751.

Thurman, D.J., Beghi, E., Begley, C.E., Berg, A.T., Buchhalter, J.R., Ding, D., Kroner, B., 2011. Standards for epidemiologic studies and surveillance of epilepsy. Epilepsia 52, 2–26. https://doi.org/10.1111/j.1528-1167.2011.03121.x.

Usmani, A., Mujahid, M.D., Khushtar, M., Siddiqui, H.H., Rahman, M.D.A., 2016. Hepatoprotective effect of Anacyclus pyrethrum Linn. against antitubercular drug-induced hepatotoxicity in SD rats. J. Complement. Integr. Med. 13, 295–300.

Vezzani, A., French, J., Bartfai, T., Baram, T.Z., 2011. The role of inflammation in epilepsy. Nat. Rev. Neurol. 7, 31–40. https://doi.org/10.1038/nrneurol.2010.178.

Yoo, K.M., Kim, K.S., Cho, M.Y., Park, B.C., 1996. Effects of kainic acid-induced seizures on c-fos protein expression in the rat hippocampus. J. Korean. Neurol. Assoc. 14, 102–113.

Zaidi, S.M.A., Pathan, S.A., Singh, S., Jamil, S., Ahmad, F.J., Khar, R.K., 2013. Anticonvulsant, anxiolytic and neurotoxicity profile of Aqarqarha (*Anacyclus pyrethrum*) DC (compositae) root ethanolic extract. Pharmacol. Pharm. 4, 535–541. https://doi.org/10.4236/pp.2013.47077.

Zhang, X.M., Zhu, J., 2011. Kainic Acid-induced neurotoxicity: targeting glial responses and glia-derivedcytokines. Curr. Neuropharmacol. 9, 388–398. https://doi.org/10.2174/157015911795596540.

Zheng, X.Y., Zhang, H.L., Luo, Q., Zhu, J., 2011. Kainic acid-induced neurodegenerative model: potentials and limitations. J. Biomed. Biotechnol. 2011, 457079. https://doi.org/10.1155/2011/457079.