

## Research Article

# Effects of *Dendrobium Officinale* Polysaccharides on Brain Inflammation of Epileptic Rats

Lin Zhang, Hua Peng, Jin Xu, Yixin Xu, You Yin, Bin He, and Jianhua Zhuang 

Department of Neurology, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China

Correspondence should be addressed to Jianhua Zhuang; [jianhuazh11@126.com](mailto:jianhuazh11@126.com)

Lin Zhang and Hua Peng contributed equally to this work.

Received 11 October 2018; Accepted 7 November 2018; Published 5 February 2019

Guest Editor: Mingqiang Li

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**Objective.** To investigate the effects of *Dendrobium officinale* polysaccharides (DOPS) on the expression of inflammatory factors IL-1 $\beta$  and TNF- $\alpha$  and the MKP-1/MAPK signal pathway. **Methods.** PTZ-induced epileptic rat models were established. The rats were randomly divided into four groups: the control group, the DOPS group, the model group, and the DOPS intervention group. RT-PCR was used to measure the mRNA expression of IL-1 $\beta$  and TNF- $\alpha$  in the hippocampi of all groups; western blot was used to measure the protein expression of IL-1 $\beta$  and TNF- $\alpha$  and phosphorylation of ERK1/2, JNK, p38, and MKP-1 in the hippocampi of all groups at weeks 1, 2, 3, and 4 after modeling. **Results.** At weeks 1, 2, 3, and 4 after modeling, there were no significant differences between the control group and the DOPS group in the mRNA and protein expression of IL-1 $\beta$  and TNF- $\alpha$  and phosphorylation of ERK1/2, JNK, p38, and MKP-1 (all  $P > 0.05$ ); the mRNA and protein expression of IL-1 $\beta$  and TNF- $\alpha$  and phosphorylation of ERK1/2, JNK, and p38 were significantly increased, while the phosphorylation of MKP-1 was decreased in the model group compared with the control group. The mRNA and protein expression of IL-1 $\beta$  and TNF- $\alpha$  and phosphorylation of ERK1/2, JNK, and p38 were significantly decreased, while the phosphorylation of MKP-1 was increased in the DOPS intervention group compared with the model group. **Conclusion.** DOPS can reduce PTZ-induced brain inflammation and seizures of epileptic rats by inhibiting IL-1 $\beta$ , TNF- $\alpha$ , and MAPK signal pathways.

## 1. Introduction

Epilepsy is one of the most common chronic brain diseases characterized by frequent recurrent seizures [1], as well as emotional and cognitive dysfunction [2]. The main cause of epilepsy is transient-distorted hypersynchronous electrical discharges of the brain network caused by imbalance between excitation and suppression [3]. Epilepsy imposes a huge burden on society and the economy, seriously affecting quality of life. It is reported that the expression of mRNA and the protein level of various proinflammatory cytokines in epileptics and rat models were increased, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 beta (IL-1 $\beta$ ) [4, 5]. Studies have reported that seizure thresholds for the overexpression of TNF- $\alpha$  were lower in transgenic rats [6]. IL-1 $\beta$  single nucleotide polymorphism was associated with temporal lobe epilepsy [7], and downregulation of the IL-1 $\beta$

signal not only delayed the onset of seizures, preventing the generalization of epilepsy, but also elevated the threshold for post-discharge induction [8]. These studies indicated that inflammatory factors played a key role in the development of epilepsy. Therefore, regulating epilepsy-related inflammatory factors is important for the treatment of epilepsy.

*Dendrobium officinale* is a kind of traditional Chinese medicine of high medicinal value, and it has anti-inflammatory, antioxidative, and immunity-enhancement effects. The main active ingredient of *Dendrobium officinale* is *Dendrobium officinale* polysaccharides, which are water-soluble and used for the treatment of epilepsy due to their strong anti-inflammatory and antioxidative effects [9]. There are few reports about the effects of DOPS on inflammatory responses induced by epilepsy and its mechanism. This study will investigate the effects of DOPS on brain inflammation in SD epileptic rats and its mechanism.

## 2. Materials and Methods

**2.1. Experimental Animals.** 96 SD male rats, about 200-220 g, were purchased from Shanghai SLAC Experimental Animal Co. Ltd. The feeding condition was at 20-25°C under 12-hour light/day, with humidity of 60%-70%, and all the rats were allowed to drink and eat freely.

**2.2. Reagent.** Pentetrazol (PTZ) was purchased from Sigma-Aldrich Trade Co. Ltd. Air-dried *Dendrobium officinale* purchased from Baise Biological Products Chain Co. Ltd. in Lingyun County, Baise, was used to prepare 0.15 g/l diluent based on Yu et al.'s method of extraction, purification, and concentration determination of DOPS [10]. IL-1 $\beta$ , TNF- $\alpha$ , and  $\beta$ -actin primers were synthesized by GenScript Biotechnology Co. Ltd. The primer sequences are seen in Table 1. Kits for total RNA extract were purchased from Shanghai Doctor Biotechnology Co. Ltd. Fluorescence quantitative PCR kits were purchased from TaKaRa Company. Rat IL-1 $\beta$  and TNF- $\alpha$  ELISA kits were purchased from Invitrogen Company. Rat p-ERK1/2, p-JNK, and p-p38 ELISA kits were purchased from RayBiotech Company. Rat p38, ERK1/2, and MKP-1 ELISA kits were purchased from LifeSpan BioSciences. Rat JNK ELISA kits were purchased from R&D Systems.

**2.3. Instruments.** The following instruments were used: NanoDrop 2000 microvolume spectrophotometers, Thermo Fisher Scientific RT-PCR system, SDS-PAGE electrophoresis system, transblot turbo transfer packs, and 3H-2000TD automatic true density analyzer.

**2.4. Animal Model Establishment.** The experimental groups consisted of the control group ( $n=24$ ), the DOPS group ( $n=24$ ), the PTZ model group ( $n=24$ ), and the DOPS intervention group ( $n=24$ ). PTZ (35 mg/kg) was given to rats in the PTZ model group and the DOPS intervention group by intraperitoneal injection; equivalent normal saline (35 mg/kg) was given to rats in the control group and the DOPS group by intraperitoneal injection. The degree of seizure was assessed by changes in behaviour of the rats within 30-50 min after injection: no seizure: no convulsion; mild seizures: facial clonus, convulsory nodding, or foreleg myoclonus; and severe seizures: hindlimb spasticity or aggravated foreleg clonus or falls due to lack of balance occurring on the basis of mild seizures. It took 4 weeks for modeling. Rats with severe seizures for a week were PTZ kindling models, and those failing to kindle were considered as failure.

**2.5. Medications.** Rats in the DOPS group and the DOPS intervention group were perfused with DOPS (1.5 g/kg) 1 hour before each intraperitoneal injection; rats in the control group and the DOPS group were perfused with equivalent normal saline and administrated for 4 weeks. Rats' behaviour during modeling was observed. Rats of each group were weighed before modeling, after the model establishment, and at weeks 1, 2, 3, and 4 after drug administration. SD rat hippocampi, 6 for each group, were isolated at low temperatures

TABLE 1: IL-1 $\beta$ , TNF- $\alpha$ , and  $\beta$ -actin primers.

Primers	Primer sequences 5'-3'
IL-1 $\beta$	5'-AGAGTGTGGATCCCAAACAA-3'
	5'-AGT CAA CTA TGT CCC GAC CA-3'
TNF- $\alpha$	5'-CTCCAGCTGGAAGACTCCTCCCAG-3'
	5'-CCC GACTACGTGCTCCTCACC-3'
$\beta$ -Actin	5'-TCAGGTCATCACTATCGGCAAT-3'
	5'-AAAGAAAGGGTGTAAAACGCA-3'

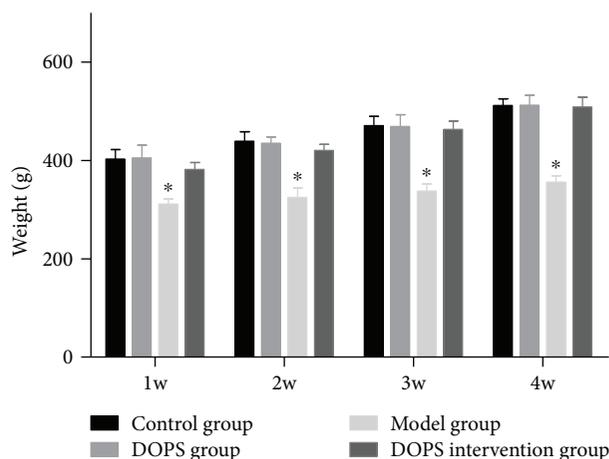


FIGURE 1: The weights of each group. \* $P < 0.05$  compared with the control group.

at weeks 1, 2, 3, and 4 after drug administration, and fresh hippocampi were stored in liquid nitrogen containers.

**2.6. RT-PCR.** Relative quantification was used to determine mRNA levels of IL-1 $\beta$  and TNF- $\alpha$ . An appropriate amount of hippocampi was obtained. The hippocampi were grinded and homogenized for subsequent RNA extraction. The extraction was guided by TRIzol kit instructions. O.D. value was measured to quantify RNA concentration, and then reverse transcription experiments were conducted with its product cDNA stored at -20°C. RT-PCR experiments were performed by using cDNA as a template according to the instructions of fluorescence quantitative PCR kits, and quantitative analysis was performed by using 2- $\Delta\Delta$ ct.

**2.7. ELISA.** Hippocampi were obtained, with PBS buffer at a ratio of 10.0 ml buffer/1.0 g tissue slices added. The brain tissue was homogenized on ice and kept on ice for 1 h. Then the brain tissue was centrifuged in a 15 ml centrifuge tube at 2000 rpm for 20 min. The supernatant was transferred to a new centrifuge tube for ELISA analysis.

**2.8. Analysis Methods.** Measurement data for each group were expressed as mean  $\pm$  standard deviation. Comparisons were based on  $t$ -tests.  $P < 0.05$  indicated statistical significance.

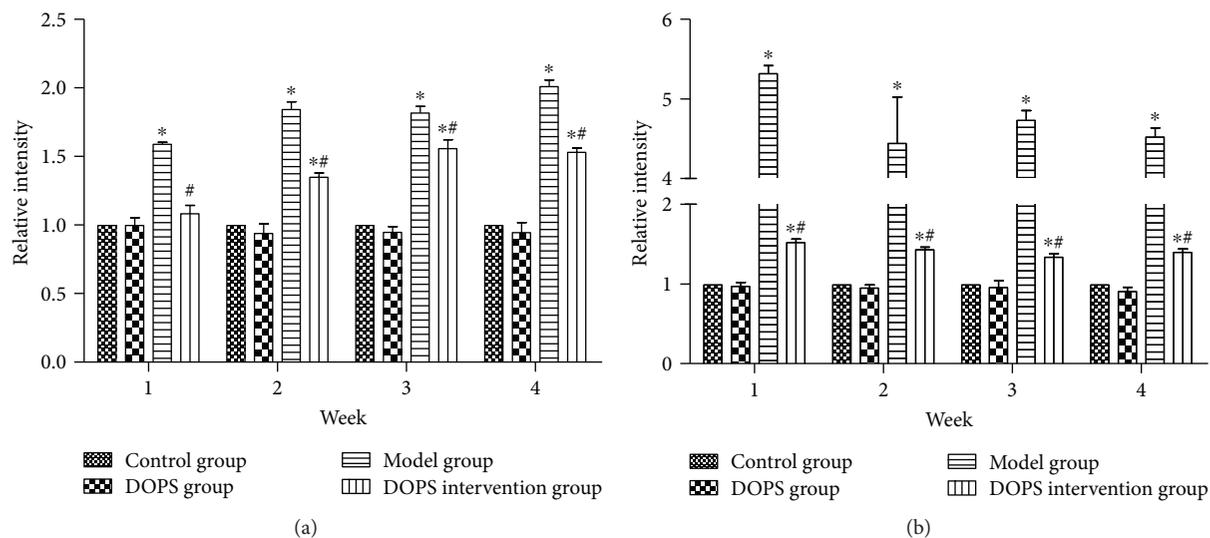


FIGURE 2: mRNA expression detection of IL-1β and TNF-α. (a) The mRNA transcription detection results of IL-1β. (b) The mRNA transcription detection results of TNF-α. \*P < 0.05 compared with the control group and #P < 0.05 compared with the model group.

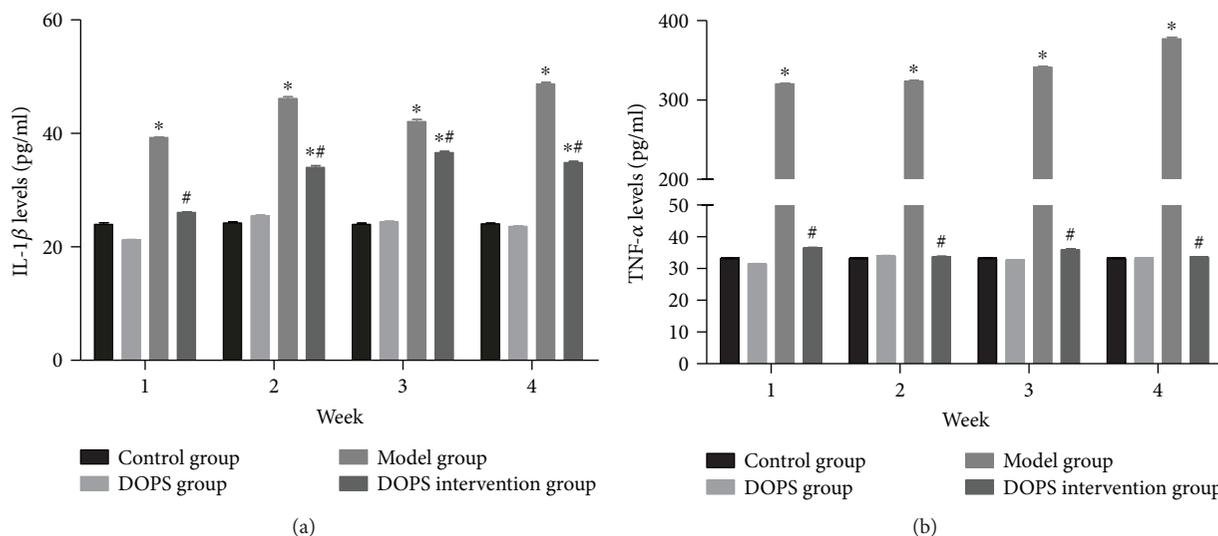


FIGURE 3: The levels of IL-1β and TNF-α by ELISA: (a) the levels of IL-1β; (b) the levels of TNF-α. \*P < 0.05 compared with the control group and #P < 0.05 compared with the model group.

### 3. Results

**3.1. Behavioural Observation.** Rats in the control group and the DOPS group had normal appetite, shiny hair, and no seizures. Rats in the PTZ model group had aggravating seizures, decreased appetite, and lost luster in hair within days after injection. Most rats developed severe seizures and overreacted to external sounds often with their bodies curled up one week after PTZ injection. Rats in the DOPS intervention group developed severe seizures two weeks after PTZ injection; however, they regained normal appetite and shiny hair with less overreactions to external sounds under continuous DOPS intervention.

**3.2. Weight.** The weights of each group are shown in Figure 1. The weights of each group were gradually increased. There were no significant differences in weights

between the control group and the DOPS group at each time point. The weights of the model group at each time point were significantly different from those of the control group. The weights of the DOPS intervention group grew faster than those of the model group and had no significant differences with those of the control group.

**3.3. mRNA Expression Detection of IL-1β and TNF-α.** There were no significant differences between the DOPS group and the control group in the levels of transcription of IL-1β and TNF-α (all P > 0.05). The levels of transcription of IL-1β and TNF-α in the model group were significantly higher than those in the control group (all P < 0.05). The levels of transcription of IL-1β in the DOPS intervention group were significantly higher than those in the control group at weeks 2, 3, and 4 (all P < 0.05), and the levels of TNF-α in the DOPS intervention group were significantly

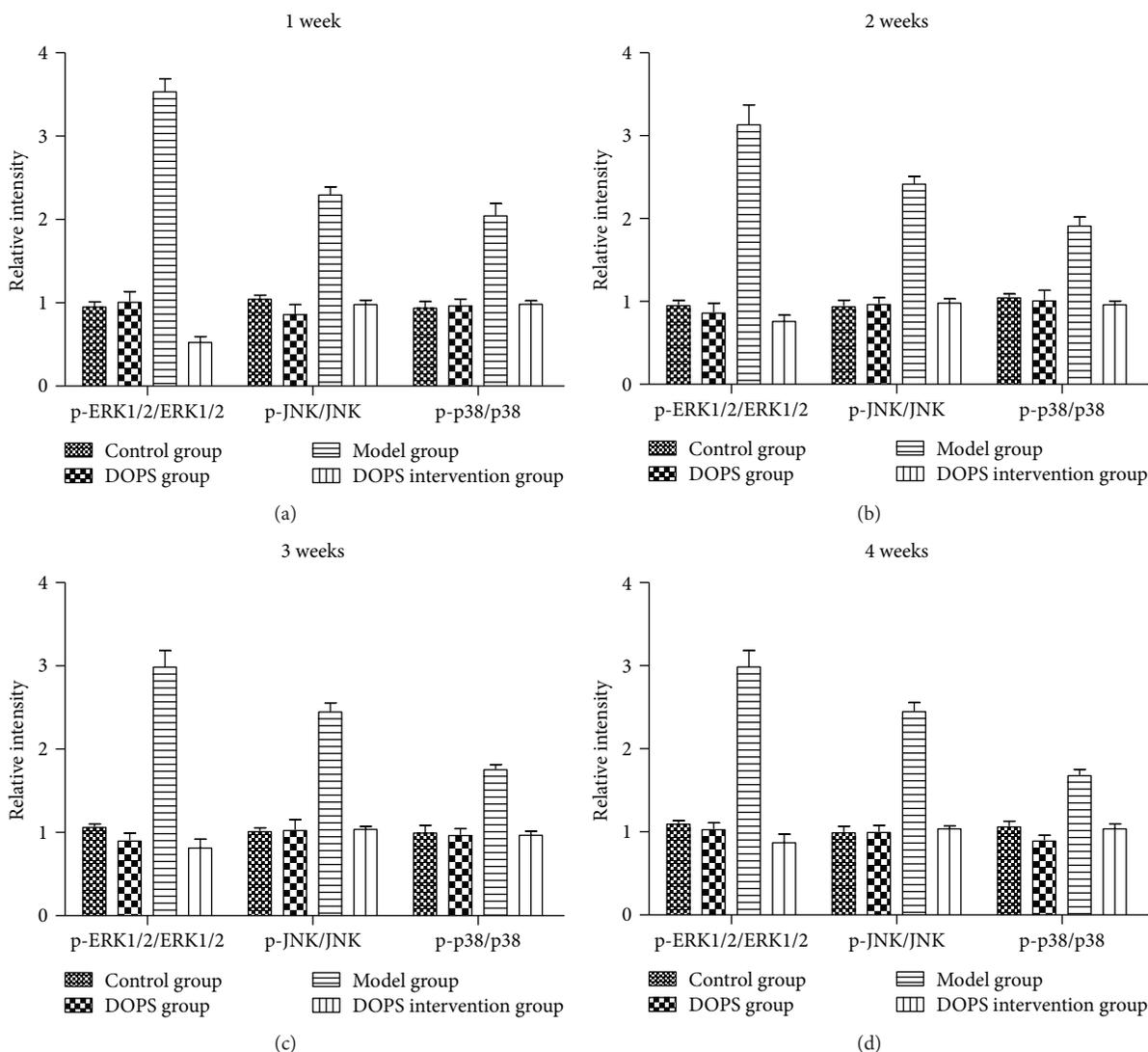


FIGURE 4: The quantitative analysis of the signaling of p-ERK1/2/ERK1/2, p-JNK/JNK, p-P38/P38. \* $P < 0.05$  compared with the control group and # $P < 0.05$  compared with the model group.

higher than those in the control group at each time point (all  $P < 0.05$ ). At each time point, the levels of transcription of IL-1 $\beta$  and TNF- $\alpha$  in the DOPS intervention group were significantly lower than those in the model group (all  $P < 0.05$ ). See Figure 2. The data further suggests that DOPS intervention can alleviate PTZ-induced neuroinflammation.

**3.4. Protein Expression Detection of IL-1 $\beta$  and TNF- $\alpha$ .** There were no significant differences between the DOPS group and the control group in levels of translation of IL-1 $\beta$  and TNF- $\alpha$  at each time point (all  $P > 0.05$ ). The levels of translation of IL-1 $\beta$  and TNF- $\alpha$  in the model group were significantly increased at each time point (all  $P < 0.05$ ). The levels of translation of IL-1 $\beta$  in the DOPS intervention group were significantly higher than those in the control group at weeks 2, 3, and 4 (all  $P < 0.05$ ), and the levels of TNF- $\alpha$  in the DOPS intervention group were significantly higher than those in the control group at each time point (all  $P < 0.05$ ). At each time point, the levels of translation of IL-1 $\beta$  and

TNF- $\alpha$  in the DOPS intervention group were significantly lower than those in the model group (all  $P < 0.05$ ). See Figure 3. The data further suggests that DOPS intervention can alleviate PTZ-induced neuroinflammation.

**3.5. MKP-1/MAPK Signal Pathway in PTZ Model Rats Was Inhibited by DOPS.** Detection found no differences between the four groups in total protein content of ERK1/2, JNK, and p38. Phosphorylation of ERK1/2, JNK, and p38 in the model group was significantly higher than that in the control group (all  $P < 0.05$ ) and was significantly higher than that in the DOPS intervention group (all  $P < 0.05$ ) (Figure 4). This suggests that the activation of the MAPK signal pathway in PTZ-induced brain dendrobium candidum was reduced by DOPS.

MKP-1 can dephosphorylate MAPK and inhibit the activity of ERK, JNK, and p38 in stress responses, thereby participating in the regulation of inflammatory responses; thus, MKP-1 was detected. The result showed that

phosphorylation of MKP-1 in the DOPS intervention group was higher than that in the model group (Figure 5). It suggests that MKP-1 activation is involved in the inhibition of MAPK intervened by DOPS.

#### 4. Discussion

Epilepsy is a common neurological disease, yet its pathogenesis has not been fully understood; however, studies have shown that seizures may be associated with immune dysfunction in patients, and it was proposed to treat immune dysfunction in epileptics [11]. Studies indicated that seizures could be induced by oxidative stress injury. And a large number of oxygen free radicals could cause inflammatory reactions, synthesize and release many inflammatory factors in the brain, and activate the NF- $\kappa$ B pathway and the MAPK signal pathway [12]. Studies have shown that DOPS could reduce oxidative products to improve the antioxidant capacity and correct the oxidative/antioxidative imbalance [13]. DOPS can inhibit the NF- $\kappa$ B pathway and release of proinflammatory factors and enhance the activity of antioxidant enzymes in the blood [14]. In this study, DOPS had no effect on the growth and survival of normal SD rats, and there were no significant differences in weight gain between the DOPS group and the control group.

The NF- $\kappa$ B pathway controls the synthesis and release of such downstream inflammatory cytokines as IL-1 $\beta$ , TNF- $\alpha$ , and IL-10 during the development of epilepsy. IL-1 $\beta$  and TNF- $\alpha$  are proinflammatory cytokines released by brain inflammation-activated microglia, and PTZ and traumatic injuries can stimulate the production of IL-1 $\beta$  and TNF- $\alpha$  in the brain [15, 16]. The study found that DOPS can inhibit the expression of IL-1 $\beta$  and TNF- $\alpha$  transcription and translation of PTZ-induced rat hippocampi. Studies have shown that inhibition of IL-1 $\beta$  and TNF- $\alpha$  expression could reduce the effects of T lymphocytes and monocytes. Excessive proinflammatory cytokines produced by activated microglia were toxic to neurons [17, 18]. The inhibition of IL-1 $\beta$  and TNF- $\alpha$  expression in the study suggests that DOPS can reduce PTZ-induced seizures by regulating microglia-mediated inflammatory responses.

Pathological mechanisms such as inflammation, ischemia, and apoptosis are related to the regulation of the MAPK signal pathway [19]. MAPK consists of three major subsets, ERK1/2, JNK, and p38, and plays a key role in transmitting various extracellular signals to nuclei and regulating cell growth and differentiation. The expression of proinflammatory cytokines is under the control of various transcription factors and regulation of the MAPK signal pathway [20]. The result of the study indicates that DOPS can inhibit the MAPK signal pathway by reducing phosphorylation of ERK1/2, JNK, and p38 in vivo. This suggests that DOPS can enhance ERK1/2, JNK, and p38 dephosphorylation. MKP-1 is another member of the MAPK family that enables dephosphorylation and inactivation of various members of the family [21]. MKP-1 deficiency is known to enhance phosphorylation of p38 and JNK [22]. The study found that the level of MKP-1 phosphorylation was increased in rats given DOPS intervention. The data indicates that DOPS

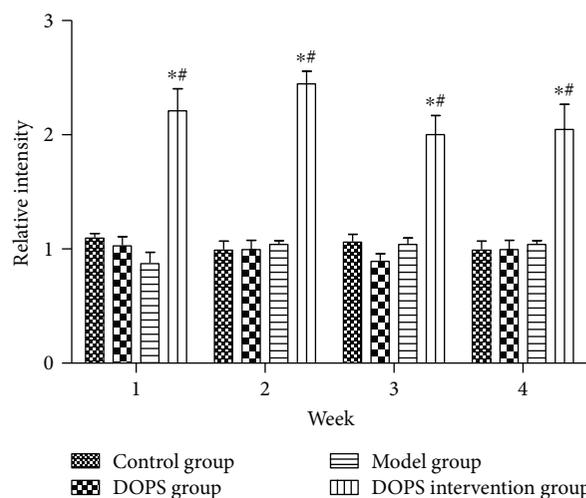


FIGURE 5: The quantitative analysis of p-MKP-1/MKP-1 signaling. \* $P < 0.05$  compared with the control group and # $P < 0.05$  compared with the model group.

blocked the MAPK signal pathway by enhancing MKP-1 phosphorylation.

In summary, DOPS can reduce the effects of PTZ-induced brain inflammation in epileptic rats as well as seizures by inhibiting the IL-1 $\beta$ , TNF- $\alpha$ , and MAPK signal pathway.

#### Data Availability

All the data is available in the handwritten notebook documented in our lab.

#### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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