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Original Article

The Effect of Kaempferol on Cortical NLRP3/Caspase-1/GSDMD-mediated Cell Pyroptosis in Chronic Epileptic Rats

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ABSTRACT

Aim: To study the Kaempferol (Kaem) on Pentetrazol (PTZ)-induced chronic epileptic rats, and to explore its effect on nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3)/caspase-1/gasdermin D (GSDMD)-mediated pyroptosis in the cerebral cortex and its possible mechanism. **Materials and Methods**: Six rats were randomly selected from 30 SPF male SD rats as the normal group, and the remaining rats were intraperitoneally injected with PTZ (35 mg/kg) for 28 d to replicate the chronic epilepsy model. The 18 successfully modeled rats were randomly divided into model, low-dose Keam (Kaem-L), Middle-dose Keam (Keam-M) and high-dose Kaem (Kaem-H) groups, with 6 rats in each group. The rats in Kaem-L, Keam-M and Kaem-H groups received 1, 2 and 10 mg/kg of Kaem by continuous lavage for 14 d, while those in normal and model groups were given equal volume of saline. The latency of Generalized Tonic-Clonic Seizures (GTCS) and Minimal Clonic Seizures (MCS) was recorded. The pathological changes of the temporal lobe cortex were observed by HE staining. Neuronal apoptosis was detected by TUNEL staining. The protein levels of NLRP3, caspase-1 and GSDMD in the cortex were detected by immunohistochemical staining and Western blot. The expression levels of TNF-α, interleukin-18 (IL-18) and IL-1β in brain tissue were detected by immunohistochemical staining. The mRNA expression levels of NLRP3, caspase-1 and GSDMD in the cerebral cortex were detected by RT-qPCR. **Results**: Compared with normal group, the latency of GTCS and MCS in the rats of model group was significantly shortened ($p<0.01$). Compared with normal group, the number of apoptotic cortical neurons in model group was significantly increased. The protein expression levels of TNF-α, IL-18 and IL-1β in the cortical tissues were significantly increased, and the mRNA and protein expression levels of NLRP3, caspase-1 and GSDMD were also significantly increased ($P<0.01$). Compared with model group, the latency of GTCS and MCS in Kaem treated groups was significantly prolonged, the number of apoptotic cortical neurons in rats was significantly reduced, the protein expression levels of TNF-α, IL-18 and IL-1β in cortical tissue was reduced to varying degrees, and the mRNA and protein expression of NLRP3, caspase-1 and GSDMD also showed varying degrees of reduction ($p<0.01$). **Conclusion**: The Kaem may reduce the secretion of inflammatory factors and inhibit the pyroptosis of nerve cells through the NLRP3/caspase-1/GSDMD signaling pathway, thus playing a therapeutic role in chronic epileptic rats induced by PTZ.

Keywords: Epilepsy, Kaem, NLRP3/caspase-1/GSDMD signaling pathway, Pyroptosis.

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INTRODUCTION

Epilepsy, as a common chronic neurological disease in clinical practice, is caused by intermittent high synchronization abnormal neuronal discharge in the brain, leading to long-term recurrent seizures in patients, accompanied by cognitive and behavioral disorders. Approximately 30% of patients develop resistance to antiepileptic drugs (ASDs) in clinical practice, and some patients do not achieve effective relief after treatment with ASDs. Research has shown that neuroinflammation plays an increasingly important role in the occurrence and development of epilepsy.[1] Inflammatory reactions can cause abnormal neuronal discharge, thereby disrupting neural connections and leading to neuronal damage and glial cell proliferation.[2] And studies have shown that during the pathological process of epilepsy, nerve cells undergo pyroptosis, which increases the secretion of pro-inflammatory cytokines and further activates the neuroinflammatory response.[3] The classic pathway of cell pyroptosis is the activation of pro-caspase-1 to caspase-1 by inflammasomes. Caspase-1 can promote cytokine maturation and release mature cytokines from the protein pore formed at the N-terminus of GSDMD by cleaving

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GSDMD, leading to neuroinflammation and driving neuronal pyroptosis. Nucleotide bound oligomeric domain like receptor protein 3 (NLRP3) is an inflammasome that plays an important role in the pathogenesis of epilepsy. It is composed of NLRP3, apoptotic associated spot like protein (ASC) containing caspase recruitment domains, and phosphorylated caspase-1 precursors. Research has shown that the activation of NLRP3 inflammasomes can promote the secretion of pro-inflammatory cytokines, leading to the occurrence of neuroinflammation,[4,5] while the transcription and expression of NLRP3/caspase-1/GSDMD pathway proteins.[6] Kaempferol is a commonly used traditional Chinese medicine. Modern pharmacological research has found that Siegesbeckia herb contains flavonoids, which have biological activities such as anti-inflammatory, antioxidant, and antibacterial properties.[7] Several flavonoids have been reported to improve neurological function by increasing the phosphorylation levels of the cyclic adenosine monophosphate responsive element binding protein (CREB)/brain-derived neurotrophic factor (BDNF) pathway.[8] But so far, there has been no evidence of the therapeutic effect of Kaempferol (Kaem) on epilepsy. This experiment established a chronic epilepsy rat model using Pentylenetetrazol (PTZ) to investigate the therapeutic effect of Kaem on chronic epilepsy rats, and further explore the effect and possible mechanism of Kaem on NLRP3/caspase-1/GSDMD mediated cell apoptosis.

MATERIALS AND METHODS

Animal

60 SPF grade 8-week-old male SD rats with a body weight of (250±10) g were provided by Liaoning Changsheng Biotechnology Co., Ltd., Rats are raised at a temperature of 20-24°C and a humidity of 50%~60%. They are kept in a standard environment for one week and are free to eat and drink water.

Main reagents

Kaempferol from Sigma; PTZ (Cool Chemical Technology Co., Ltd., batch number: GT633714); TUNE detection kit (Roche, batch number: 10768100); NLRP3, and pro caspase-1 polyclonal antibodies (Wuhan Doctoral Biotechnology Co., Ltd., product numbers: BA3677, and BM4291); Anti GSDMD-N antibody (Beijing Anolun Biotechnology Co., Ltd., product number: DF13758); Anti caspase-1 p20, GSDMD, tumor necrosis factor alpha (TNF - α), interleukin 18 (IL-1 β and GAPDH polyclonal antibodies, and goat anti rabbit HRP labeled IgG (Beijing Boersen Biotechnology Co., Ltd., product numbers: bs-10743R, bs-14287R, bs-10802R, bs-0529R, bs-0812R, bs-0061R, and bs-0296G-HRP); TRIpure Reagent, SuperM-MLV reverse transcriptase, RNase inhibitor, and 2x Power Taq PCR Master Mix (purchased by Beijing Keam from Sigma Company; Baitaike Biotechnology Co., Ltd., product numbers: RP1001, PR6502, RP5602, and PR1702); SYBR Green qPCR Master Mix (Beijing Solebao Technology Co. Ltd., item number: SY1020).

Main instruments

DNM-9602 enzyme-linked immunosorbent assay (Beijing Planck New Technology Co., Ltd.,); Positive fluorescence microscope FR-4A (Shanghai Optical Instrument Factory); YD-1508B tissue slicer (Zhejiang Jinhua Yidi Medical Equipment Factory); Motic BA400 Microscopic Imaging System (Motic); ICEN-24R desktop high-speed freezing centrifuge (Hangzhou Aosheng Instrument Co., Ltd.,); Tanon EPS-300 electrophoresis apparatus, Tanon VE-186 electrophoresis tank, Tanon VE-180B membrane transfer tank and Tanon-4600 gel imaging system (Shanghai Tianneng Technology Co., Ltd.,); Exocler 96 fluorescence quantitative PCR instrument (BIONEER).

Main methods

preparation and evaluation Model

51 rats were adaptively fed for 7 days and numbered and weighed. The model group rats were intraperitoneally injected with PTZ solution (35 mg/kg) daily for 28 days.[9] After injection, quickly place the rats in a transparent box for observation. This study monitored the duration and intensity of epileptic seizures in each group of rats after the last administration, and determined the seizure level of the rats according to the Racine score standard:[10] 0 level, with no seizure response; Grade I, with symptoms such as chewing and rhythmic facial twitching; Grade II, with symptoms of chewing and nodding of the head accompanied by more severe facial muscle twitching; Grade III, with unilateral forelimb spasms but not accompanied by upright posture; Grade IV, with bilateral forelimb spasms accompanied by upright posture; Grade V, experiencing ankylosing convulsions, standing upright or falling all over the body; Level VI, death. 18 rats were successfully modeled based on at least 3 consecutive epileptic seizures with severity not lower than grade IV occurring within 28 days.

Grouping and administration methods

36 successfully prepared rats were randomly divided into a model group, a low-dose Kaem (Kaem L) group, and a high-dose Kaem (Kaem H) group, with an additional normal group consisting of six rats in each group. Referring to reference,[11] rats in the Kaem-L and Kaem-H groups were given Kaem solution at doses of 1 and 10 mg/kg by gavage, once daily for 14 consecutive days. The normal group and model group were given equal volumes of physiological saline by gavage every day.

Generalized Tonic-Clonic Seizures (GTCS) and Minimal Clonic Seizures (MCS) latency

After each intraperitoneal injection of PTZ, the GTCS latency and MCS latency of each group of rats were observed according to the literature[12] and Racine grading criteria.

Table 1: The sequences of the primer for RT-qPCR.

Gene Name	F:(5'-3')	R:(5'-3')
Caspase-1	AGCTTCAGTCAGGTCCATCAGC	GGCAAAACTTGAGGGAACCAC
NLRP3	TCCCGCATCTCGATTTGT	GCTGGGTGTAGCGTCTGT
GSDMD	TGAAGATCGTGGATCATGCC	GGTAGAATTCCGAAGGCAGT
GAPDH	AGATCATCAGCAATGCCTCCT	TGAGTCCTTCCACGATACCAA

HE staining observation of pathological changes

Take brain tissue from each group of rats and fix it with 4% paraformaldehyde for 24 hr. After routine dehydration, transparency, waxing, and paraffin embedding, slice and perform HE staining. Observe the pathological changes in the temporal lobe cortex brain tissue of each group of rats under a microscope.

Observation of cell apoptosis using TUNEL method

According to the instructions of the TUNEL detection kit, observe the apoptosis of neurons in the cerebral cortex area under a light microscope. Take 6 discontinuous slices from each group of rats for detection, calculate the percentage of neuronal apoptosis in 3 different fields of view for each slice, and take the average value for statistical analysis.

Immunohistochemistry (IHC) detection of protein expression in cortical tissues of rats in each group

The PV two-step method was used for detection, and the paraffin sections were subjected to routine dewaxing and hydration followed by microwave repair; Deactivate endogenous enzymes and clean with PBS; Dropwise addition of I antibodies (NLRP3, GSDMD-N, IL-18, IL-1 β, and TNF - α antibodies, 1:125); caspase-1 p20 antibodies (1:100), incubated overnight at 4 °C; After PBS cleaning, add II antibody dropwise and incubate at 37 °Cfor 30 min; DAB staining and hematoxylin double staining; After dehydration and transparency, seal the film. 9 cases were selected for testing in each group, and images were collected under 200x magnification using the Motic3000 micrographic imaging system. Three different fields of view were randomly selected from each slice, and Image Pro Plus was used Using image analysis software, the protein expression level is represented by the average Integrated Absorbance (IA).

Western Blotting detection of NF - κ B (*p65*), NLRP3, GSDMD, GSDMD-N, pro caspase-1, and caspase-1 p20 protein expression in the temporal lobe cortex of rats in each group

Weigh cortical brain tissue samples, grind them on ice, lyse them with RIPA cell lysate, centrifuge with a freeze centrifuge, and take the supernatant for quantification using BCA protein quantification method. Use metal bath denaturation as a backup. After successful preparation of the protein sample, electrophoresis separation was performed, and a constant current of 200 mA was transferred onto a PVDF membrane. After being blocked with skim milk powder for 2 hr, the samples were incubated overnight at 4 °C in rabbit anti NLRP3, pro caspase-1, caspase-1p20, GSDMD, and GSDMD-N (all 1:1000). Add goat anti rabbit HRP labeled IgG (1:2000) and incubate at room temperature for 1 hr, then perform ECL chemiluminescence development, using GAPDH as the internal reference. Use ImageJ software to quantitatively analyze each group of bands, calculate the ratio of the grayscale value of the corresponding band of the target protein to the grayscale value of the internal reference band, and determine the expression level of the target protein.

RT-qPCR detection of mRNA expression of NLRP3, GSDMD, and caspase-1 in the cortex of rats in each group

Take fresh cortical tissue, extract total RNA, and use NanoDrop 2000 UV spectrophotometer to measure the concentration of RNA in each sample. Reverse transcribe the RNA samples obtained above to obtain the corresponding cDNA. PCR amplification was performed using SYBR GreenMaster Mix under reaction conditions of 94 °C for 5 min; 94 °C for 10 sec, 60 °C for 20 sec, 72 °C for 30 sec, cycle 40 times. Using GAPDH as an internal reference, the results were analyzed for relative expression levels using $2^{-\Delta\Delta Ct}$. The synthetic primer sequence of Shanghai Shenggong is shown in Table 1.

Statistical processing

Using SPSS 25 Perform statistical analysis using 0 software. Normally distributed econometric data are represented as mean±standard deviation (mean±SD). Inter group mean comparison was conducted using one-way analysis of variance, and pairwise comparison was conducted using LSD-*t* test. $p<0$ 05 indicates a statistically significant difference.

RESULTS

The effect of Kaem on the latency of GTCS and MCS in PTZ induced epileptic rats

During the experiment, the Normal group did not show any symptoms of epileptic seizures; Compared with the Model group, the incubation periods of GTCS and MCS in all Keam treatment groups were significantly prolonged ($p<0.01$, Figure 1). Meanwhile, there were significantly differences among Keam-L, Keam-M and Keam-H groups ($p<0.05$).

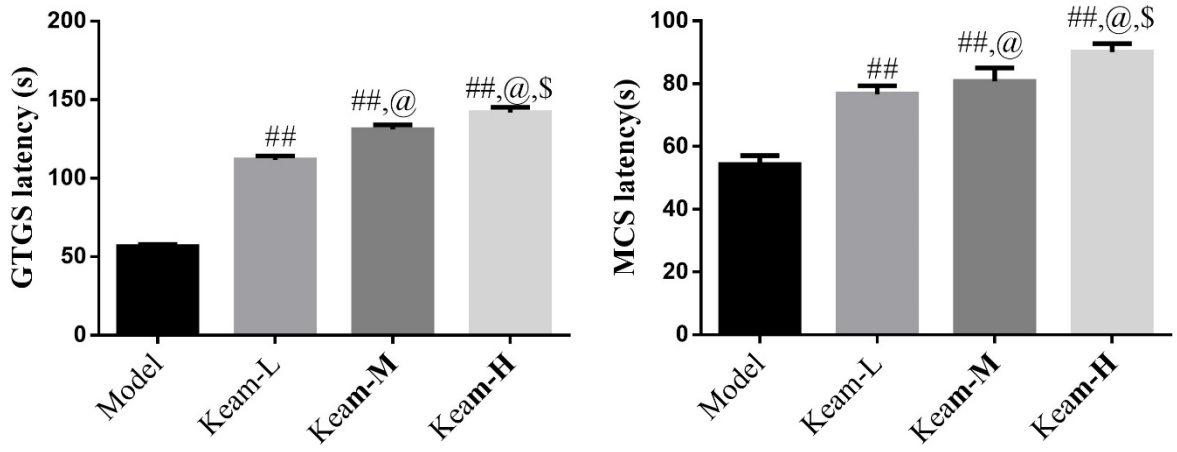

Figure 1: Comparison of GTCS and MCS latency of rats in each group.

##: $p<0.01$, compared with Model group; @: $p<0.05$, compared with Keam-L group; $: $p<0.05$, compared with Keam-M group

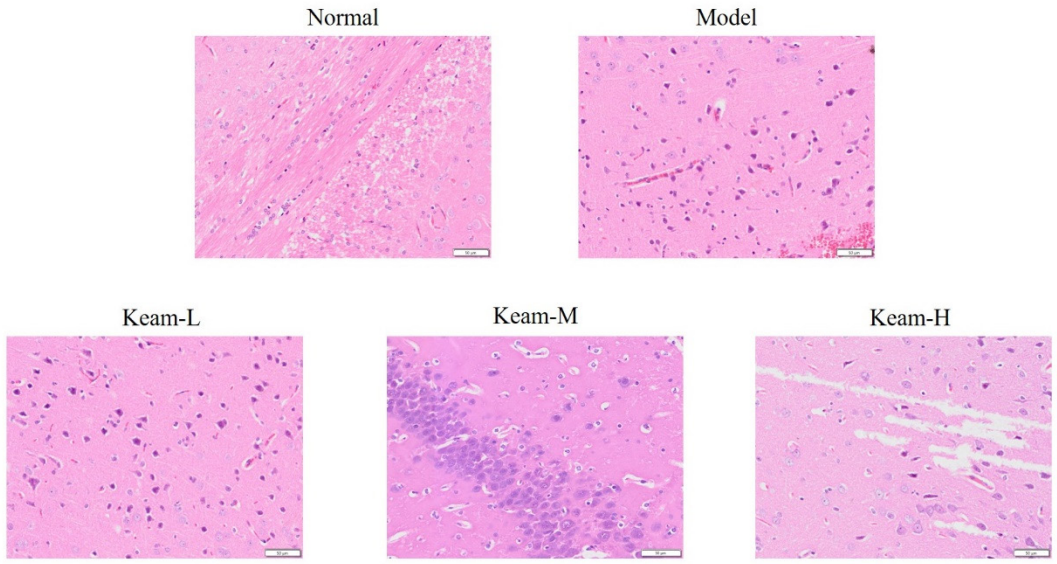

Figure 2: HE staining was used to detect neuronal damage in the cortex of rats in each group (scale bar=50 μm).

The effect of Keam on the pathological morphology of brain tissue in PTZ induced epileptic rats

The brain tissue structure of the Normal group rats is clear, with no edema in the tissue, regular arrangement of nerve cells, uniform staining of cytoplasm and nuclei, clear nucleoli, and no congestion or inflammatory cell infiltration; Compared with the normal group, the brain tissue structure of the Model group rats was loose, the morphology of nerve cells was irregular, nuclear pyknosis was significantly increased, cell swelling and deep staining were observed, microglia and inflammatory cells were significantly increased, and capillaries were congested and dilated; Compared with the model group, the above-mentioned lesions in the cortex of rats in each Keam group were alleviated to varying degrees, manifested by a denser brain tissue structure, more uniform staining, reduced number of nuclear pyknosis, reduced number of inflammatory cells and microglia, reduced capillary congestion, and more significant reduction in pathological changes in the high-dose group, as shown in Figure 2.

The effect of Kaem on PTZ induced neuronal apoptosis in epileptic rats

The TUNEL staining results showed that compared with the Normal group, the Model group significantly increased the number of neuronal apoptosis in rats ($P<001$); Compared with the Model group, the number of neuronal apoptosis in the Keam-L, Keam-M and Keam-H groups of rats was significantly reduced ($p<0.05$) 01, Figure 3). Meanwhile, there were significantly differences among Keam-L, Keam-M and Keam-H groups ($p<0.05$).

The effect of Keam on the expression of NLRP3, Caspase-1 p20, and GSDMD-N proteins in the brain tissue of PTZ induced epileptic rats

The IHC results showed that the expression of NLRP3, caspase-1 p20, and GSDMD-N proteins was lower in the brain tissue of the Normal group rats; Compared with the Normal group, the expression of NLRP3, caspase-1 p20, and GSDMD-N proteins in the brain tissue of the Model group rats was significantly increased (p<0.01), respectively, Figure 4), Western cells are brownish yellow in color; Compared with the Model group, the expression of NLRP3, caspase-1 p20, and GSDMD-N proteins in the brain tissue of the Keam treatment group was significantly reduced (p<0.01), respectively, Figure 4). Meanwhile, there were significantly differences among Keam-L, Keam-M and Keam-H groups (p<0.05).

The effect of Keam on the expression of IL-1 β, IL-18, and TNF - α proteins in the brain tissue of PTZ induced epileptic rats

The IHC results showed that the expression of IL-1 β, IL-18, and TNF - α proteins was lower in the brain tissue of the Normal group rats; Compared with the Normal group, the expression of IL-1 β, IL-18, and TNF - α proteins in the brain tissue of the Model group rats was significantly increased (p<0.01), respectively, Figure 5), Western cells are brownish yellow in color; Compared with the Model group, the expression of IL-1 β, IL-18, and TNF - α proteins in the brain tissue of the Keam treatment group was significantly reduced (p<0.01), respectively, Figure 5). Meanwhile, there were significantly differences among Keam-L, Keam-M and Keam-H groups (p<0.05).

The effect of Kaem on the expression of NF - κ B (o65), NLRP3, pro caspase-1, Caspase-1 p20, GSDMD, and GSDMD-N proteins in the brain tissue of PTZ induced epileptic rats

Western blotting results showed that compared with the Normal group, the expression levels of NLRP3, pro caspase-1, Caspase-1 p20, GSDMD, and GSDMD-N proteins in the Model group were significantly increased (p<0.01), respectively, Figure 6). The protein expression levels of NLRP3, pro caspase-1, Caspase-1 p20, GSDMD, and GSDMD-N were significantly reduced in the Keam treatment group (p<0.01), respectively, Figure 6). Meanwhile, there were significantly differences among Keam-L, Keam-M and Keam-H groups (p<0.05).

The effect of Keam on the mRNA expression of NLRP3, Caspase-1, and GSDMD in PTZ induced epileptic rat brain tissue

The RT qPCR results showed that compared with the Normal group, the mRNA expression levels of NLRP3, caspase-1, and GSDMD in the brain tissue of the Model group rats were significantly increased (P<0.01), respectively, Figure 7); Compared with the Model group, the mRNA expression levels of NLRP3, caspase-1, and GSDMD in the brain tissue of each Keam treatment group were significantly reduced (p<0.01), respectively, Figure 7). Meanwhile, there were significantly differences among Keam-L, Keam-M and Keam-H groups (p<0.05).

DISCUSSION

Epilepsy is a complex clinical syndrome. The International Anti Epilepsy Alliance believes that neuronal death is the susceptibility basis for recurrent seizures, and residual neurons and proliferative glial cells form abnormal neural networks, which can lead to further development of epilepsy.[13] Neuroptosis is related to the occurrence and development of epilepsy. During the process of neuroptosis, a large amount of pro-inflammatory cytokines is released, activating inflammasomes and causing a cascade of neuroinflammatory reactions. The NLRP3 inflammasome can dephosphorylate and activate phosphorylated caspase-1, leading to the transformation of pro-inflammatory cytokines into their mature forms. At the same time, activated caspase-1 binds to GSDMD and cleaves it. Cytokines are released from the protein pores formed by GSDMD-N, further activating the NLRP3 inflammasome.[14] GSDMD is a substrate of inflammatory caspases and a promoter of pyroptosis.[15] The cytokines IL-1 β and IL-18 released from the N-terminus of GSDMD protein can promote pyroptosis.[16] The excessive release of inflammatory mediators can activate specific signaling pathways such as NMDA receptors and Toll like receptors to participate in neuronal excitation, leading to sustained progression of seizures.[17,18]

The results of this experiment showed that compared with the normal group, the expression of TNF - α, IL-18, and IL-1 β proteins in the brain tissue of epilepsy model rats was significantly increased, and the expression of NLRP3, caspase-1 p20, GSDMD protein and mRNA were also significantly increased. This indicates the occurrence of neuronal pyroptosis in the brain tissue of chronic epileptic rats induced by PTZ, accompanied by the release of a large number of pro-inflammatory cytokines. The activation of NLRP3 inflammasomes exacerbates the inflammatory cascade reaction, thereby further promoting the occurrence and development of pyroptosis.

Cellular pyroptosis is one of the important pathological mechanisms of epilepsy, and how to effectively inhibit cell pyroptosis by regulating the activation of NLRP3 inflammasomes is the key to the prevention and treatment of epilepsy. Aminoflavonoids can inhibit the activation of NLRP3 inflammasomes in PTZ induced mouse epilepsy models and reduce the levels of inflammatory cytokines. Research has shown that NF - κ B can activate pyroptosis signaling factors such as NLRP3, ASC, GSDMD, caspase-1, and IL-1 β to promote cell pyroptosis.[19,20]

Keam has anti-inflammatory and inhibitory pharmacological effects on cell apoptosis, such as berberine which can also

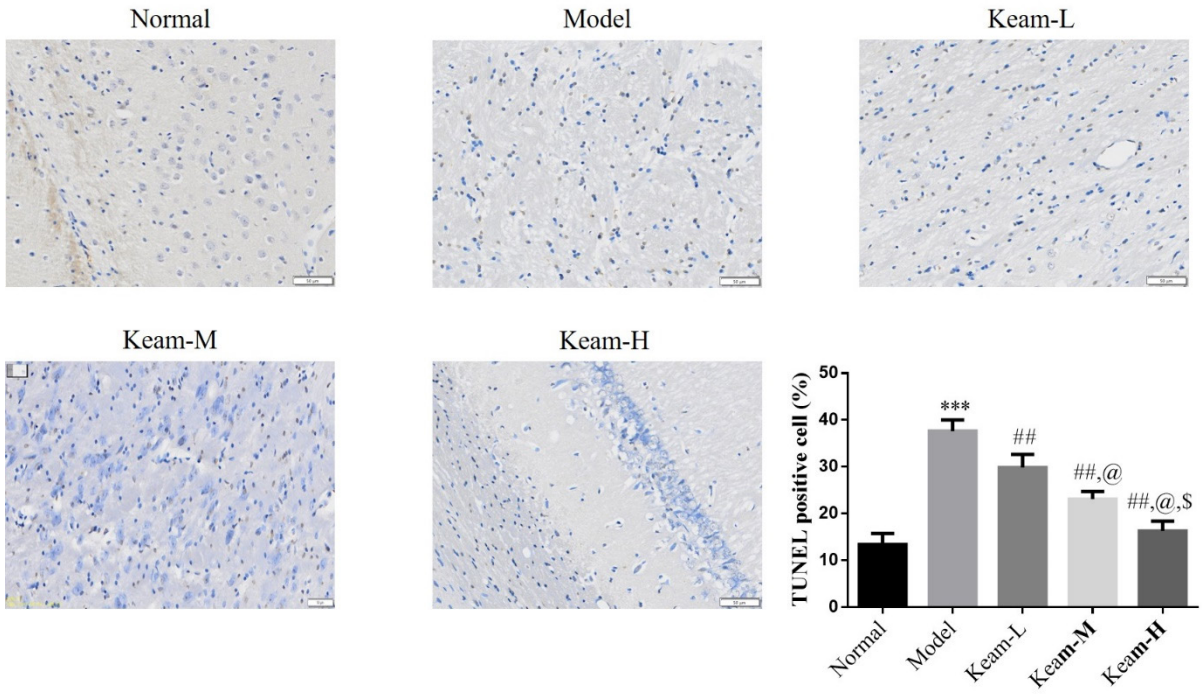

Figure 3: Expression of TUNEL positive cells in the cortex of rats in each group (scale bar=50 μm).

**: $p<0.01$, compared with Normal group; ##: $p<0.01$, compared with Model group; @: $p<0.05$, compared with Keam-L group; $: $p<0.05$, compared with Keam-M group.

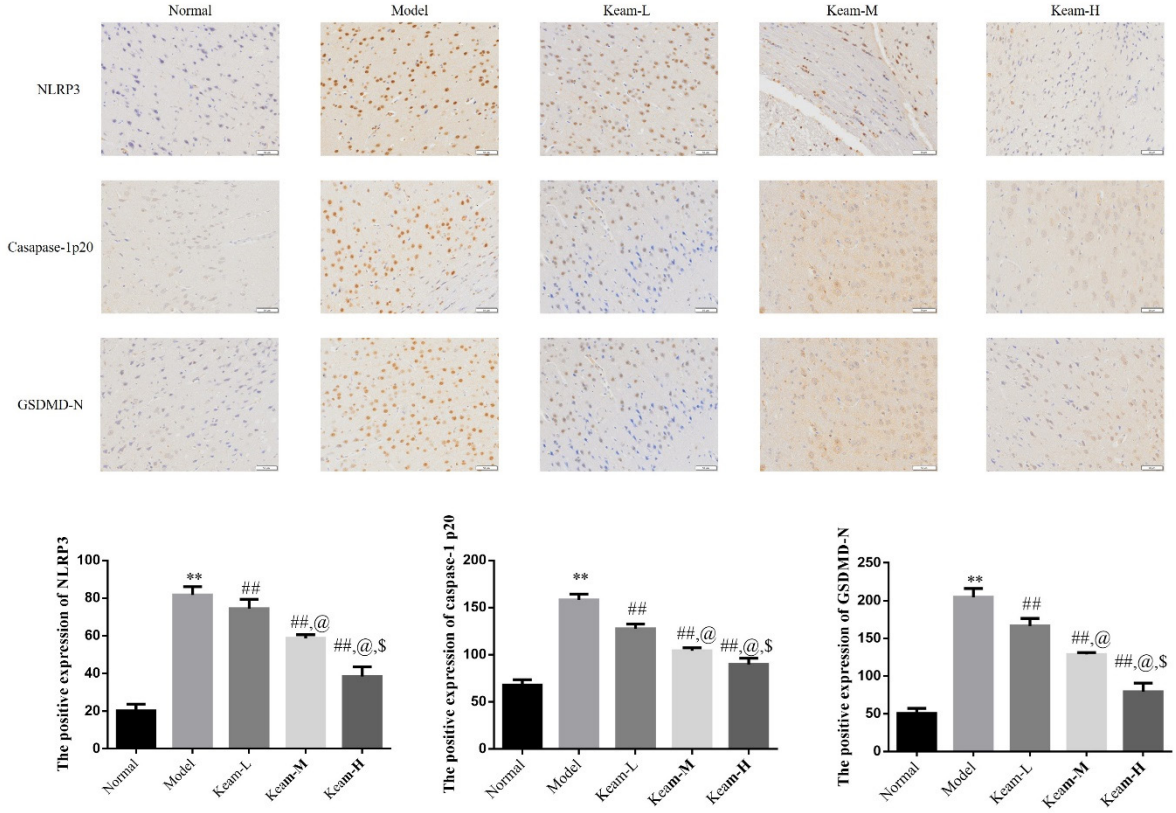

Figure 4: The positive expression of NF-κB p65, NLRP3, caspase-1 p20 and GSDMD-N in the cortex was detected by immunohistochemical staining (scale bar=50 μm).

**: $p<0.01$, compared with Normal group; ##: $p<0.01$, compared with Model group; @: $p<0.05$, compared with Keam-L group; $: $p<0.05$, compared with Keam-M group.

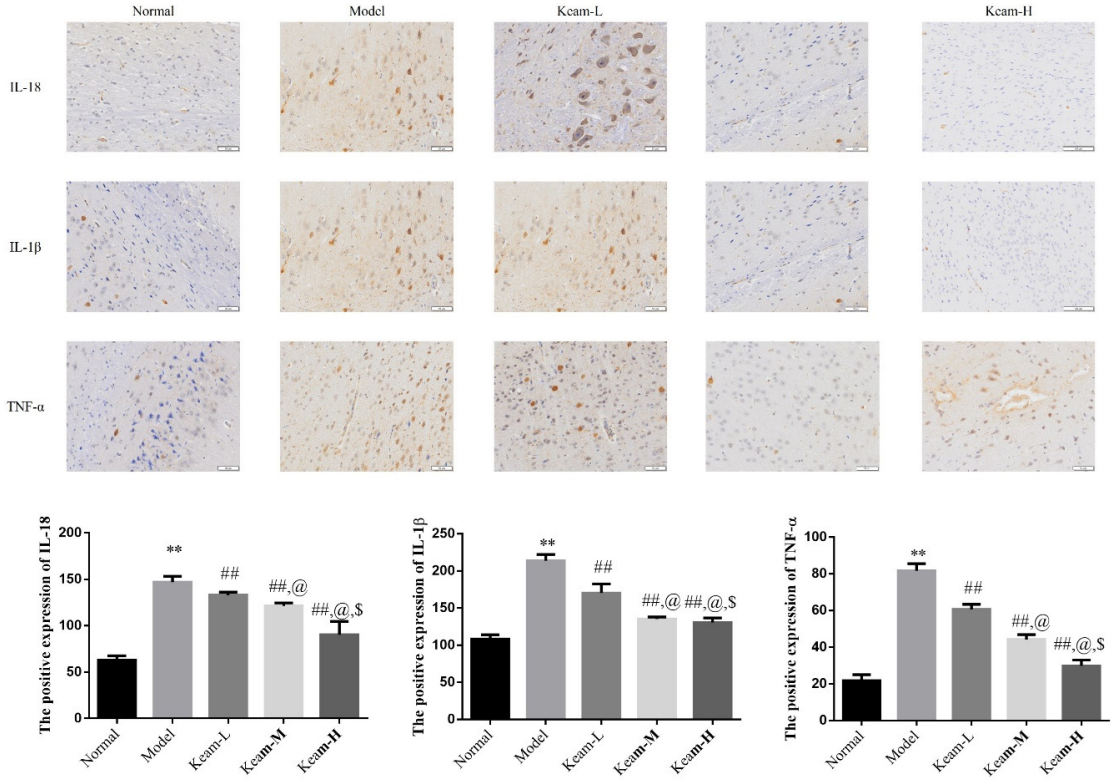

Figure 5: The positive expression of IL-18, IL-1β and TNF-α in the cortex was detected by immunohistochemical staining (scale bar=50 μm).

**: $p<0.01$, compared with Normal group; ##: $p<0.01$, compared with Model group; @: $p<0.05$, compared with Keam-L group; $: $p<0.05$, compared with Keam-M group.

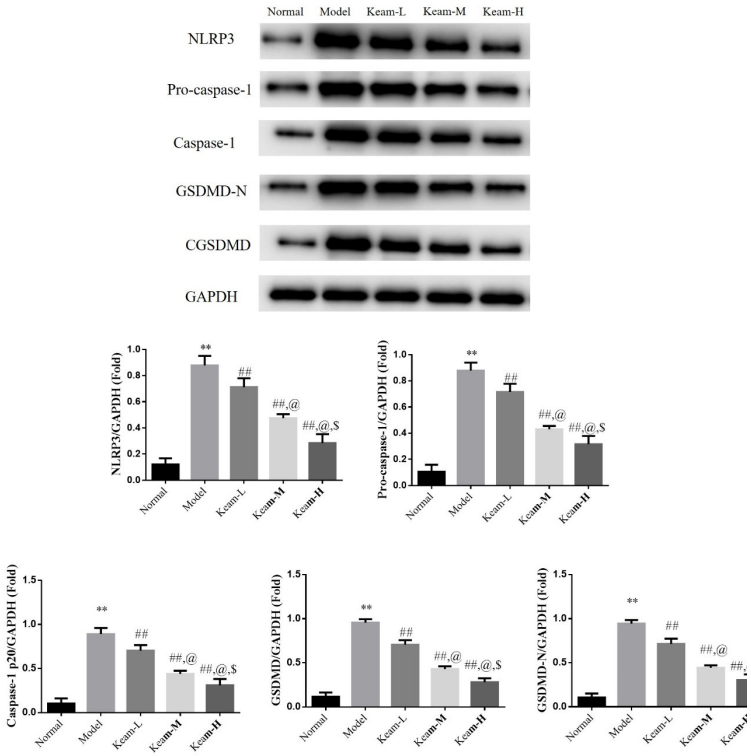

Figure 6: Protein expression of NF-κB, NLRP3, Pro-caspase-1, caspase-1, GSDMD and GSDMD-N in the cortex of rats in each group detected by Western blotting (scale bar=50 μm).

**: $p<0.01$, compared with Normal group; ##: $p<0.01$, compared with Model group; @: $p<0.05$, compared with Keam-L group; $: $p<0.05$, compared with Keam-M group.

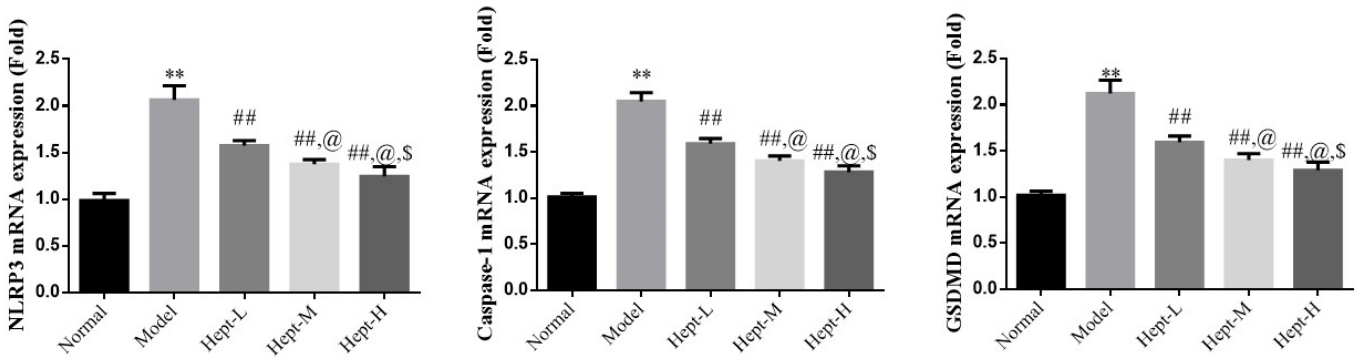

Figure 7: Relative mRNA expression of NLRP3, caspase-1 and GSDMD in the cortex of rats in each group detected by RT-qPCR.

**: $p<0.01$, compared with Normal group; ##: $p<0.01$, compared with Model group; @: $p<0.05$, compared with Keam-L group; $: $p<0.05$, compared with Keam-M group

inhibit the production of TNF-α.[21] Keam can effectively alleviate cervical spondylotic radiculopathy in rats through the NLRP3 inflammatory pathway.[22] The results of this experiment showed that Keam can significantly reduce the frequency and intensity of epileptic seizures in model rats, alleviate neuronal pathological damage caused by PTZ, and have a significant therapeutic effect on epileptic rats. This suggests that Keam may block the activation of NLRP3 inflammasomes, inhibit the activation of GSDMD and caspase-1, reduce the release of IL-1 β and IL-18 inflammatory cytokines, and thus alleviate neuronal pyroptosis caused by epilepsy. Meanwhile, Keam also inhibits the inflammatory cascade in brain tissue through the Nlrp3 signaling pathway, further inhibiting the progression of neuronal pyroptosis. However, further determination is needed to determine whether Keam plays a role in regulating the Nlrp3 mediated pyroptosis signaling pathway. Depending on our present study, the results found High concentration (10 mg/kg) of Keam had strong effects.

However, there were some limits in our research, like as, we did not conduct *in vitro* study to discuss the mechanism, and due to time and financial constraints, it is not possible to conduct a long-term study this time. This deficiency will be addressed in future research.

CONCLUSION

Kaem inhibits the neuroinflammatory cascade through the NLRP3/caspase-1/GSDMD signaling pathway, further inhibiting neuronal pyroptosis, and thus has a significant therapeutic effect on PTZ induced chronic epilepsy in rats.

CONLFICTS OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NLRP3: Nucleotide-binding oligomerization domain-like receptor protein 3; **GSDMD:** Gasdermin D; **GTCS:** Generalized tonic-clonic seizures; **MCS:** Minimal clonic seizures; **CREB:** Cyclic adenosine monophosphate responsive element binding protein; **BDNF:** Brain-derived neurotrophic factor.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by Ethics Committee of Tangshan Vocational and Technical College Affiliated Hospital.

SUMMARY

Keam is an extraction from herb, in our present study, we used vivo study to discuss Keam's treatment effects to Chronic Epileptic Rats via regulation NLRP3/Caspase-1/GSDMD pathway.

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