Electroacupuncture attenuates chronic fibromyalgia pain through the phosphorylated phosphoinositide 3-kinase signaling pathway in the mouse brain

Chao-Tsung Chen 1, Jaung-Geng Lin 2, Chun-Ping Huang 3, Yi-Wen Lin 3*

1 Center for General Education, Chung Yuan Christian University, Taoyuan 32023, Taiwan
2 College of Chinese Medicine, School of Chinese Medicine, China Medical University, Taichung 40402, Taiwan
3 College of Chinese Medicine, Graduate Institute of Acupuncture Science, China Medical University, Taichung 40402, Taiwan

Introduction

Fibromyalgia (FM) is a disabling disease that manifests as chronic, widespread nociceptive sensations. Patients experience spreading pain accompanied by fatigue, depression, memory problems, anxiety, sleep disturbances, and headaches. These symptoms impair the daily functioning of FM patients. The prevalence of FM is 2%-8% of the population (1, 2). For an unknown reason, FM occurs more frequently in females. FM is known to affect all ethnicities and ages, even presenting in children. Due to its chronic nature, FM requires long-term management. However, as the mechanism of action of FM is poorly understood, effective treatments are lacking. The pathogenesis of FM is puzzling and limited research is available to identify the etiology. Given that a chronic, long-term illness like FM can debilitate an individual, it is hoped that better understanding of the mechanism of FM can be used to develop more effective treatment strategies (3, 4).

Phosphoinositide 3-kinase (PI3K) belongs to a family of enzymes involved in cell growth, proliferation, and differentiation. PI3K can activate the AKT signaling pathway for cell proliferation and survival. The PI3K pathway is stimulated to protect astrocytes from apoptosis (5). PI3K can be subdivided into PI3Kα, PI3Kβ, PI3Kδ, and PI3Kγ. PI3Kδ, PI3Kβ, and PI3Kγ which are all activated by receptor tyrosine kinases, which are transmembrane glycoproteins with enzymatic activity. In addition, PI3Kγ, can be activated by both G-protein-coupled receptors and Ras (6). Activation of PI3Ks can convert phosphatidylinositol 4,5-biphosphate (PIP2) into phosphatidylinositol 3,4,5-trisphosphate (PIP3) to phosphorylate Akt (7, 8). This activation will then turn on the mTOR signaling pathway and further activate NFκB for transcription (9).

Acupuncture is a traditional technique that has been used for over 3,000 years to treat conditions such as stroke (10), dementia (11), and pain (12-15). Analgesia by acupuncture is generally accepted worldwide. The first study to demonstrate the analgesic effect of acupuncture was published in 1973 (16). More recent studies have shown that the analgesic effect of acupuncture is mediated by the release of endogenous opiates (17) and serotonin (18). Endogenous opiate concentrations in plasma have been shown to increase in response to acupuncture (19) or increase as neurotransmitters in the cerebrospinal fluid (20).

Our rationale for the current study is that electroacupuncture (EA) is effective for treating FM pain with unclear mechanisms. We hypothesized that EA could effectively treat mice with chronic FM-associated mechanical hyperalgesia by regulating the Phosphoinositide 3-kinase (PI3K) pathway. The PI3K signaling pathway is involved in a more effective transmembrane glycoproteins with enzymatic activity. In addition, PI3Kγ, can be activated by both G-protein-coupled receptors and Ras (6). Activation of PI3Ks can convert phosphatidylinositol 4,5-biphosphate (PIP2) into phosphatidylinositol 3,4,5-trisphosphate (PIP3) to phosphorylate Akt (7, 8). This activation will then turn on the mTOR signaling pathway and further activate NFκB for transcription (9).

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pain model (12-15). Accordingly, we aimed to identify whether EA attenuated mechanical hyperalgesia in a mouse model of chronic FM. We also verified whether the PI3K signaling pathway was involved in the peripheral and central nervous systems.

Materials and Methods

Animals

We used 8–12 week old C57BL/6 mice purchased from BioLASCO Co Ltd (Taipei, Taiwan) for all experiments. The mice were randomly assigned to four groups (n= 8 per group): (1) Normal (controls), (2) FM, (3) FM+2 Hz EA, and (4) FM+sham EA. The sample size required for an alpha of 0.05 and a power of 80% was six animals per group. Mice were housed in a room under a 12/12 hr light/dark cycle with ad libitum access to water and food. All procedures were approved by the Institutional Animal Care and Use Committee of China Medical University (No. 2018-110) and conducted in accordance with the Guide for the Use of Laboratory Animals provided by the National Research Council and the ethical guidelines of the International Association for the Study of Pain. The number of animals used and their suffering were minimized.

FM induction and mechanical hyperalgesia measurement

All mice except controls received a 20 μl injection of acidic saline (pH 4.0) into the right gastrocnemius muscle (IM) under isoflurane (1%) anesthesia on day 0. The second acidic saline injection was administered on day 5 to establish the FM mouse model. The acidic saline was prepared in 10 mM 2-[N-morpholino]ethanesulfonic acid (MES) and further adjusted to pH 4.0 with 1 N NaOH. Mechanical sensitivity was tested at weeks 2 and 3 after the first acidic saline injection. Mechanical sensitivity was measured by testing the strength of responses to stimulation with von Frey filaments (North Coast Medical, Gilroy, CA, USA). Mice were placed on a metal mesh and adapted to the new environment for at least 30 min before testing.

Acupuncture manipulation

EA was administered in the morning (9:00–10:00 am) starting 2 weeks after the induction of FM. EA treatment lasted for 15 min at a frequency of 2 Hz and an amplitude of 1 mA and was performed three times per week for 2 weeks. EA was delivered using stainless steel needles (0.5 inch, 32G, Yukuang, Taipei, Taiwan) vertically inserted into the muscle layer at a depth of 3–5 mm in the ST36 acupuncture point under 1% isoflurane anesthesia. The ST36 acupuncture point is located on the tibialis anterior muscle, approximately 1/6 of the distance from the patella to the lateral malleolus. In the FM + sham EA group, a needle was inserted into the ST36 acupuncture point under 1% isoflurane anesthesia without any rotation or twisting.

Tissue sampling and Western blot analysis

After the treatment period, mice were sacrificed and the tibialis anterior muscle, spinal cord (SC), full thalamus, and somatosensory cortex (SSC) neurons were immediately excised for protein extraction. Total proteins were prepared by homogenizing the tissues in lysis buffer containing 50 mM Tris-HCl (pH 7.4), 250 mM NaCl, 1% NP-40, 5 mM EDTA, 50 mM NaF, 1 mM Na3VO4, 0.02% NaN03, and 1× protease inhibitor cocktail (Amresco, Solon, OH, USA). The extracted proteins (30 μg per sample, according to the BCA protein assay) were subjected to 8% sodium dodecyl sulfate-Tris glycine gel electrophoresis and transferred to a PVDF membrane. The membrane was blocked with 5% non-fat milk in TBS-T buffer (10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween 20), incubated with primary antibodies against pI3K (~125 kDa, 1:1000, Millipore, Bedford, MA, USA), pAkt (~60 kDa, 1:1000, Millipore), pmTOR (~60 kDa, 1:500, Millipore), and pNFκB (~65 kDa, 1:1000, Millipore) in TBS-T and 1% bovine serum albumin, and incubated for 1 hr at room temperature. A peroxidase-conjugated anti-rabbit antibody (1:5000) was used as the secondary antibody. The bands were visualized using an enhanced chemiluminescent substrate kit (Pierce, Rockford, IL, USA) with LAS-3000 Fujifilm (Fuji Photo Film Co Ltd, Tokyo, Japan). Where appropriate, the image intensities of specific bands were quantified using NIH ImageJ software (Bethesda, MD, USA). The protein ratios were determined by dividing the target protein intensities by the intensity of α-tubulin or β-actin in the same sample. The calculated ratios were adjusted by dividing the ratios from the same comparative group relative to controls.

Statistical analysis

All data are expressed as mean±standard error. Differences between the normal, FM, 2 Hz EA, and sham EA groups were tested by analysis of variance followed by a Post hoc Tukey’s test. The level of statistical significance was P<0.05.

Results

Induction of chronic FM pain and further attenuation by a 2 Hz EA manipulation, but not sham treatment

A similar mechanical threshold was observed in the normal, FM, 2 Hz EA, and sham operated groups under basal conditions (Figure 1). Significant mechanical hyperalgesia was observed in the FM, 2 Hz EA, and sham EA groups two weeks after inducing FM. Mechanical hyperalgesia was maintained in the FM mice during week 4. In addition, 2 Hz EA treatment reliably attenuated mechanical hyperalgesia. Attenuation was not observed in the sham EA group, suggesting the specificity of EA.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Mechanical withdrawal thresholds in each group of mice. Normal saline injection (Normal group, n=8). CFM (Acid saline-induced FM pain), 2 Hz EA (Acid saline-induced FM pain treated with 2 Hz EA), and sham EA (Acid saline-induced FM pain treated with sham EA). *P<0.05 vs. Normal group. **P< 0.05 vs. CFM group
Chronic FM pain did not alter expression of the PI3K signaling pathway in the mouse peripheral DRG or central SC

Western blot was used to quantify PI3K protein levels and sequential molecules in the mouse DRG. We confirmed the presence of pPI3K in normal mouse DRG (Figure 2A, 100.1±7.2%, P>0.05, n=8). Chronic FM induction did not alter expression (Figure 2A, 88.5±6.5%, P>0.05, n=8), and potentiation of pPI3K was not altered by 2 Hz or sham EA (Figure 2A, 90.2±5.2% and 92.3±3.8%, P>0.05, n=8). We then tested if downstream pAkt shared similar mechanisms with pPI3K. The results showed that pAkt was unchanged in chronic FM mice (Figure 2B, 93.3±3.2%, P>0.05, n=8). Similarly, expression was similar in the 2 Hz EA and sham control groups (Figure 2B, 93.6±4.2% and 95.8±3.6%, P>0.05, n=8). Next, we quantified the expression of pmTOR, a downstream pAkt factor, in the mouse DRG. We found that pmTOR was unchanged in chronic FM mice (Figure 2C, 92.6±10.2%, P>0.05, n=8), and that expression was similar in the 2 Hz EA and sham control groups (Figure 2C, 92.1±12.1% and 92.2±12.4%, P>0.05, n=8). Furthermore, pNFκB was unaltered in FM mice (Figure 2D, 114.9±14.8%, P>0.05, n=8), and expression was similar in the 2 Hz EA and sham control groups (Figure 2D, 109.3±11.2% and 112.5±13.7%, P>0.05, n=8). We also determined that PI3K, pAkt, pmTOR, and pNFκB

**Figure 2.** Expression levels of pPI3K-associated signaling pathway proteins in the mice lumbar DRG. (A) pPI3K, (B) pAKT, (C) pmTOR, and (D) pNFκB expression levels in tissues from the Normal, CFM, CFM + 2 Hz EA, and CFM + sham EA (from left to right). Normal = normal mice; CFM = chronic fibromyalgia mice; 2Hz EA = CFM + 2 Hz EA, Sham EA = CFM + sham EA. *P<0.05 compared with the normal group. #P<0.05 vs. CFM group. The Western blot bands at the top show the target protein. The lower bands are internal controls (β-actin or α-tubulin).

**Figure 3.** Expression levels of pPI3K-associated signaling pathway proteins in the mice lumbar SC. (A) pPI3K, (B) pAKT, (C) pmTOR, and (D) pNFκB expression levels in tissues from the Normal, CFM, CFM + 2 Hz EA, and CFM + sham EA (from left to right). Normal = normal mice; CFM = chronic fibromyalgia mice; 2Hz EA = CFM + 2 Hz EA, Sham EA = CFM + sham EA. *P<0.05 compared with the normal group. #P<0.05 vs. CFM group. The western blot bands at the top show the target protein. The lower bands are internal controls (β-actin or α-tubulin).
Chronic FM pain increases expression of the PI3K signaling pathway in the mouse central thalamus and SSC

We then examined PI3K protein expression in the central thalamus to determine if the PI3K signaling pathway is involved in central sensitization of chronic FM pain. Our data confirmed the presence of PI3K in the mouse thalamus and showed that the level was increased in the chronic FM group (Figure 4A, 100.1 ± 9.2% and 129.6 ± 12.3%, *P < 0.05, n=8). Potentiation was reversed by 2 Hz EA stimulation, but not in the sham operated group (Figure 4A, 90.9 ± 7.4% and 129.6 ± 11.7%, *P < 0.05, n=8). Furthermore, our results showed augmentation of pAkt in the thalamus of FM mice (Figure 4B, 138.4 ± 6.7%, *P < 0.05, n=8), which was attenuated by 2 Hz EA in FM mice (Figure 4B, 104.2 ± 12.6%, *P < 0.05, n=8) but not in the sham control group (Figure 4B, 131.1 ± 7.7%, *P < 0.05, n=8). Similar results were observed for pmTOR level in the FM (Figure 4C, 141.8 ± 12.8%, *P < 0.05, n=8), EA (Figure 4C, 95.3 ± 7.2%, *P < 0.05, n=8), and sham EA groups (Figure 4C, 141.8 ± 12.8%, *P < 0.05, n=8). To identify transcriptional regulation of FM and EA, we further verified NFκB expression in the mouse thalamus. We found that pNFκB was expressed in the mouse thalamus and increased in FM mice (Figure 4D, 138.6 ± 6.9%, *P < 0.05, n=8). This increase was reversed by 2 Hz EA, but not by sham EA (Figure 4A, 84.9 ± 11.5% and 158.3 ± 7.7%, *P < 0.05, n=8). We also examined the...
SSC, which is sensitive to pain, to assess whether the PI3K signaling pathway participates in this brain area. The data confirmed that pPI3K is present in the mouse SSC and is significantly increased in FM mice (Figure 5A, 140.3±11.2%, P<0.05, n=8). Again, augmentation was attenuated by 2 Hz EA, but not by sham EA (Figure 5A, 105.9±6.4% and 141.5±11.9%, P<0.05, n=8). We also found that pAkt was increased in the SSC of FM mice and could be reduced by 2 Hz EA (Figure 4A, 127.1±9.3% and 85.1±11.8%, P<0.05, n=8), but that there was no effect in the sham group (Figure 4A, 128.3±11.9%, P<0.05, n=6). Similar results were observed for pmTOR and pNFkB protein expression in the mouse SSC (Figure 5C, D).

Discussion

FM pain is strongly associated with inflammation. As demonstrated by clinical study, inflammatory mediators like interleukin (IL)-1β, IL-6, IL18, and tumor necrosis factor (TNF)α are commonly elevated in FM patients (21, 22). Ohgidani et al. reported that microglia are hypersensitive to ATP in patients with FM; TNF-α secreted from microglia appears to play a crucial role in the development of FM (23). A recent study suggested that the cyclooxygenase-2 and PI3K/Akt signaling pathways are crucial in chemotherapy-induced neuropathic pain (24). Local inflammation initiates significant central sensitization of nociceptive SC neurons, and TNF and AMPA receptors are known to be involved in this process. Pre-treatment with a PI3K inhibitor can reliably reduce potentiation of the AMPA receptor due to inflammation (25). Liu et al. reported that the PI3K-Akt signaling pathway was significantly increased in the SC of chronic constriction injury (CCI) rats. CCI also potentiates phosphorylation of the AMPA receptor and induces mechan allodynia (26). The results of the present study indicated that pPI3K-pAkt was unaltered in the peripheral DRG and central SC neurons in all groups. The increase in pPI3K-pAkt in the mouse thalamus and SSC after inducing FM could be reversed by 2 Hz EA, but there was no effect in the sham group. This is the first report demonstrating that pPI3K-pAkt is involved in chronic FM pain.

A recent study of CCI rats showed that the pain threshold decreased on days 7 and 14 after induction, whereas both mRNA and protein levels of PI3K, Akt, and mTOR increased (27). Furthermore, glial fibrillary acidic protein, which is used to mark OX-42, is simultaneously increased in the CCI group. Pritchard et al. reported that blocking PI3K signaling pathway prevents the development of acidic saline-induced hyperalgesia (28). Taken together, these results suggest that chronic FM induces significant mechanical hyperalgesia accompanied by increased expression of the PI3K signaling pathway in the mouse thalamus and SSC. These findings support the potential application of EA for managing FM pain.

Conclusion

In the present study, we first demonstrated the ability of an acidic saline injection to induce FM pain in a murine model. We then showed that mechanical hyperalgesia was attenuated by 2 Hz EA simulation, but not by sham. Notably, pPI3K, pAkt, pmTOR, and pNFkB were unaltered in the DRG and SC of chronic FM mice. These molecules were also unaltered after 2 Hz or sham EA treatments. By contrast, pPI3K, pAkt, pmTOR, and pNFkB were increased in the mouse thalamus and SSC after inducing FM. The increase in signals was attenuated by 2 Hz EA, but not by the sham EA. Taken together, these results suggest that chronic FM induces significant mechanical hyperalgesia accompanied by increased expression of the PI3K signaling pathway in the mouse thalamus and SSC. These findings support the potential application of EA for managing FM pain.

References

1. English B. Neural and psychosocial mechanisms of pain sensitivity in fibromyalgia. Pain Manag Nurs 2014; 15:530-538.
2. Clauw DJ. Fibromyalgia: a clinical review. JAMA 2014; 311:1547-1555.
3. Dadabhoy D, Crofford LJ, Spaeth M, Russell IJ, Clauw DJ. Biology and therapy of fibromyalgia. Evidence-based biomarkers for fibromyalgia syndrome. Arthritis Res Ther 2008; 10:211.
4. Panerai AE, Vecchiet J, Panzeri P, Meroni P, Scaroni S, Pizzigallo E, et al. Peripheral blood mononuclear cell beta-endorphin concentration is decreased in chronic fatigue syndrome and fibromyalgia but not in depression: preliminary report. Clin J Pain 2002; 18:270-273.
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5. Gomez Del Pulgar T, De Ceballos ML, Guzman M, Velasco G. Cannabinoids protect astrocytes from ceramide-induced apoptosis through the phosphatidylinositol 3-kinase/protein kinase B pathway. J Biol Chem 2002; 277:36527-36533.

6. Deladeriere A, Gambardella L, Pan D, Anderson KE, Hawkins PT, Stephens LR. The regulatory subunits of PI3Kgamma control distinct neutrophil responses. Sci Signal 2015; 8:ra8.

7. Rubashkin MG, Cassereau L, Bainer R, DuPortCC, Yui Y, Ou G, et al. Force engages vinculin and promotes tumor progression by enhancing PI3K activation of phosphatidylinositol (3,4,5)-triphosphate. Cancer Res 2014; 74:4597-4611.

8. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 2005; 307:1098-1101.

9. Ouyang ZH, Wang WJ, Yan YG, Wang B, Lv GH. The PI3K/Akt pathway: a critical player in intervertebral disc degeneration. Oncotarget 2017; 8:57870-57881.

10. Liao HY, Ho WC, Chen CC, Lin JG, Chang CC, Chen LY, et al. Clinical evaluation of acupuncture as treatment for complications of cerebrovascular accidents: a randomized, sham-controlled, subject- and assessor-blind trial. Evid Based Complement Alternat Med 2017; 2017:7498763.

11. Lin YW, Hsieh CL. Electroacupuncture at Bishui acupoint (GV20) reverses behavior deficit and long-term potentiation through N-methyl-d-aspartate and transient receptor potential vanilloid subtype 1 receptors in middle cerebral artery occlusion rats. J Integr Neurosci 2010; 9:269-282.

12. Liao HY, Hsieh CL, Huang CP, Lin YW. Electroacupuncture attenuates CFA-induced inflammatory pain by suppressing Nav1.8 through S100B, TRPV1, opioid, and adenosine pathways in mice. Sci Rep 2017; 7:42531.

13. Ven LT, Hsieh CL, Hsu HC, Lin YW. Targeting ASIC3 for relieving mice fibromyalgia pain: roles of electroacupuncture, opioid, and adenosine. Sci Rep 2017; 7:46663.

14. Lu KW, Hsu CK, Hsieh CL, Yang J, Lin YW. Probing the effects and mechanisms of electroacupuncture at ipsilateral or contralateral ST36-ST37 acupoints on CFA-induced inflammatory pain. Sci Rep 2016; 6:22123.

15. Liao HY, Hsieh CL, Huang CP, Lin YW. Electroacupuncture attenuates inflammatory pain by suppressing opioid and adenosine pathways in mice. Sci Rep 2017; 7:15679.

16. Andersson SA, Ericson T, Holmgren E, Lindqvist G. Electroacupuncture and pain threshold. Lancet 1973; 2:564.

17. Han JS. Acupuncture: neuropeptide release produced by electrical stimulation of different frequencies. Trends Neurosci 2003; 26:17-22.

18. Chang FC, Tsai HY, Yu MC, Yi PL, Lin JG. The central serotonergic system mediates the analgesic effect of electroacupuncture on ZUSANLI (ST36) acupoints. J Biomed Sci 2004; 11:179-185.

19. Kiser RS, Khatami MJ, Gatchel RJ, Huang XY, Bhatia K, Altshuler KZ. Acupuncture relief of chronic pain syndrome correlates with increased plasma met-enkephalin concentrations. Lancet 1983; 2:1394-1396.

20. Clement-Jones V, McLoughlin L, Tomlin S, Besser GM, Rees LH, Wen HL. Increased beta-endorphin but not met-enkephalin levels in human cerebrospinal fluid after acupuncture for recurrent pain. Lancet 1980; 2:946-949.

21. Mendieta D, De la Cruz-Aguilera DL, Barrera-Villalpando MI, Becerril-Villanueva E, Arreola R, Hernandez-Ferreira E, et al. IL-8 and IL-6 primarily mediate the inflammatory response in fibromyalgia patients. J Neuroimmunol 2016; 290:22-25.

22. Tsilioni I, Russell JJ, Stewart JM, Gleason RM, Theoharides TC. Neuroptides CRH, SP, HK-1, and inflammatory cytokines IL-6 and TNF are increased in serum of patients with fibromyalgia syndrome, implicating mast cells. J Pharmacol Exp Ther 2016; 356:664-672.

23. Ohgidani M, Kato TA, Hosoi M, Tsuda M, Hayakawa K, Hayaki C, et al. Fibromyalgia and microglial TNF-alpha: Translational research using human blood induced microglia-like cells. Sci Rep 2017; 7:11882.

24. Jiang SB, Zhang ZD, Kang LM, Wang QH, Zhang L, Chen HP. Celecoxib reverses oxaliplatin-induced neuropathic pain through inhibiting PI3K/Akt2 pathway in the mouse dorsal root ganglion. Exp Neurol 2016; 275 Pt 1:11-16.

25. Wigerblad G, Huie JR, Yin HZ, Leinders M, Pritchard RA, Koehn JF, et al. Inflammation-induced GluA1 trafficking and membrane insertion of Ca(2+) permeable AMPA receptors in dorsal horn neurons is dependent on spinal tumor necrosis factor, PI3 kinase and protein kinase A. Exp Neurol 2017; 293:144-158.

26. Liu A, Wang X, Wang H, Lv G, Li Y, Li H. Delta-opioid receptor inhibition prevents remifentanil-induced post-operative hyperalgesia via regulating GluR1 trafficking and AMPA receptor function. Exp Ther Med 2018; 15:2140-2147.

27. Guo JR, Wang H, Jin XJ, Jia DL, Zhou X, Tao Q. Effect and mechanism of inhibition of PI3K/Akt/mTOR signal pathway on chronic neuropathic pain and spinal microglia in a rat model of chronic constriction injury. Oncotarget 2017; 8:52923-52934.

28. Pritchard RA, Falk L, Larsson M, Leinders M, Sorkin LP. Different phosphoinositide 3-kinase isoforms mediate carrageenan nociception and inflammation. Pain 2016; 157:137-146.

29. Xie X, Ma L, Xi K, Zhang W, Fan D. MicroRNA-183 suppresses neuropathic pain and expression of AMPA receptors by targeting mTOR/VEGF signaling pathway. Cell Physiol Biochem 2017; 41:181-192.

30. Kwon M, Han J, Kim UJ, Cha M, Um SW, Bai SJ, et al. Inhibition of mammalian target of rapamycin (mTOR) signaling in the insular cortex alleviates neuropathic pain after peripheral nerve injury. Front Mol Neurosci 2017; 10:79.

31. Chen Q, Mo R, Wu N, Zou X, Shi C, Gong J, et al. Berberine ameliorates diabetes-associated cognitive decline through modulation of aberrant inflammation response and insulin signaling pathway in DM rats. Front Pharmacol 2017; 8:334.