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Repositioning Vinpocetine: Behavioral and Neurochemical Modulation in a Fibromyalgia-Like Model in Mice

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Abstract

Purpose: Fibromyalgia (FM) is a debilitating condition that dramatically impairs quality of life. Neuroinflammation is a plausible initiator of a cascade of events leading to aberrant neuronal excitability. Since the available treatment options of FM are limited in producing the effective results, there is an urgent need to identify novel modalities to adequately manage FM. Herein, we aimed to investigate the potential effect of vinpocetine on the perturbation of behavioral and neurobiochemical parameters of inflammation in a mouse model of FM.

Methods: FM model was induced by two intramuscular injections of acidified saline into the right gastrocnemius muscle, administered 5 days apart. Mice received either the respective vehicle, vinpocetine (4 mg/kg/day), or pregabalin (10 mg/kg/day) for 4 weeks. The study evaluated reflexive and non-reflexive pain responses, estimation of inflammatory markers in the hippocampus and spinal cord, and extracellular signal-regulated kinase (ERK) expression in the hippocampus.

Results: Both vinpocetine and pregabalin significantly improved reflexive pain response with amelioration of inflammatory and oxidative stress markers. On the other hand, vinpocetine significantly improved anxiety and depression-like behaviors compared to pregabalin alongside enhancing ERK expression.

Conclusion: The current study suggests vinpocetine as a promising therapeutic candidate for alleviating FM symptoms, potentially through its anti-inflammatory and antioxidant effects while preserving ERK expression.

Keywords: Fibromyalgia; Depression; ERK1/2; Neurodegeneration; Nociplastic pain; Vinpocetine.

1. Introduction

Fibromyalgia (FM) is characterized by widespread pain throughout the body that is not related to any specific injury **(Sarzi-Puttini et al., 2020)**. The term "nociplastic pain" has been coined to differentiate this type of pain from the classical nociceptive or neuropathic pain categories **(Kosek et al., 2021)**. Nociplastic pain arises from an altered reactivity of the nociceptive pathway rather than actual or impending tissue damage, or damage of the somatosensory system. Research suggests that both central and peripheral sensitization play a crucial role in the aberrant processing of pain in FM which augments the sensitivity to various, usually non-noxious, stimuli **(Kosek et al., 2016; 2021).**

In addition to the principal complaint of widespread pain, patients with FM often experience generalized fatigue as well as a range of neuropsychiatric manifestations that dramatically impact a person's quality of life, both physically and emotionally **(Galvez-Sánchez et al., 2019)**.

The etiopathogenesis of FM remains inconclusive. Although sound evidence claims chronic stressful life challenges as the main factor that trigger or worsen FM symptoms, there is an evident contribution of genetic factors, emotional factors, and neuro-hormonal factors that modulate the excitability of nociceptive pathways **(Sarzi-Puttini et al., 2020; Siracusa et al., 2021)**. At the cellular level, chronic activation of microglia is a crucial factor associated with altered processing of nociceptive impulses that leads to pain amplification despite absence of the original symptoms **(Muzio et al., 2021)**. These cells are also considered the main source of inflammatory mediators, in turn, the released neurotoxic mediators promote microglia activation, and a vicious cycle is created **(Coskun Benlidayi, 2019)**.

At the subcellular level, studies unravel a strong association between neurogenic inflammation and chronic pain syndromes, including nociplastic pain **(Littlejohn and Guymer, 2018)**. Findings demonstrated that FM is linked to a distinct cytokine profile that has been observed centrally and peripherally **(O'Mahony et al., 2021)**. These inflammatory mediators can modulate excitatory and inhibitory neuropeptides to maintain a state of neuronal excitability; hence, they are believed to play an eminent role in the genesis and exacerbation of FM symptoms and related comorbidities **(Coskun Benlidayi, 2019; Marino et al., 2023)**.

The impact of oxidative stress on the dysfunction of pain pathways in FM cannot be dismissed. Numerous studies have indicated a positive correlation between lipid peroxidation products and FM symptoms highlighting their potential as a valuable therapeutic target **(Cordero et al., 2013; Sánchez-Domínguez et al., 2015; Assavarittirong et al., 2022)**.

Extracellular signal-regulated kinases (ERK) regulate pivotal cellular events including proliferation, differentiation, as well as survival by connecting surface receptor signals to a vast array of executor cytoplasmic and nuclear molecules **(Lavoie et al., 2020)**. It is therefore considered as a key signaling pathway that links external signals to the transcription machine **(Wang and Mao, 2019)**. Accumulating data, both clinical and experimental, documented the down regulation of ERK signaling in brain regions involved in memory and behavioral changes in neuropsychiatric disorders including depression **(Dwivedi et al., 2001; Wang and Mao, 2019)**. However, it is still under investigation whether normalization of ERK activity could impact the antidepressant response **(Wang and Mao, 2019)**.

The standard pharmacological options available to manage FM continue to pose a challenge **(Jurado-Priego et al., 2024)**. Despite the evident role of inflammation in the pathogenesis of FM, conventional anti-inflammatory drugs are not the ideal option as they fail to control all symptoms of FM and are associated with evident long-term side effects **(Littlejohn and Guymer, 2018)**. Some members of antidepressants or antiepileptics can manage specific FM symptoms such as pain, sleep disturbance, and fatigue. However, they may not be as effective in managing tender points **(Wiffen et al., 2013)**. Therefore, it is crucial to use a combination of drugs with different mechanisms along with non-pharmacological modalities to adequately control the symptoms of FM **(Sarzi-Puttini et al., 2020)**.

Vinpocetine is an inhibitor of PDE1 that has a beneficial effect in cerebrovascular disorders. It has an established safety profile and is increasingly being used as a memory-boosting supplement. **(Zhang et al., 2018)**. Vinpocetine exerts a distinct anti-inflammatory action and antioxidant properties in a variety of cell types **(Petric et al., 2023)**. It has been also evaluated in a number of pain conditions triggered by various **insults (Lourenco-Gonzalez et al., 2019)**. However, the potential role of vinpocetine in the context of FM has yet to be

addressed.

Corroborating the multiple mechanisms of action of vinpocetine, we sought to investigate its protective effects in the pain and behavior domains of a model of FM induced by acidic saline injection. In addition, we aimed to explore whether the effect of vinpocetine was linked to central and peripheral anti-inflammatory and antioxidant effects as well as altered ERK expression in the brain.

2. Materials and Methods

2.1. Experimental animals

The experiment was conducted on forty male mice (15-20 g) recruited from the animal care unit (Alexandria Faculty of Medicine). Animals were housed at an appropriate temperature (25 ± 2 °C) and alternating cycles of light and dark by turning the light on/off at 7:00 a.m., and 7:00 p.m., respectively. The present work received the approval number: 0306041 from the "Ethics Board for the Experimental Research Studies" (Alexandria University, Alexandria, Egypt).

2.2. Experimental design

Mice were allocated randomly to four groups of 10 animals each: control group (Control), acidified saline injection (ASI)-treated group (ASI group), ASI and vinpocetine-treated group (ASI +Vinpo), ASI and pregabalin-treated group (ASI +Preg).

For induction of FM, the area of the right gastrocnemius muscle was shaved. Next, 100 µl of low-pH sterile saline (pH 4.0±0.1) was injected into the muscle using an insulin syringe. After five days, the right gastrocnemius muscle was re-injected with the same procedure. Animals of the control group received similar injection procedure but with normal saline **(Liu et al., 2014b; Álvarez-Pérez et al., 2022)**. We adopted the dose of 4 mg/kg/day of vinpocetine based on previous studies that demonstrate its neuroprotective effects **(Shekarian et al., 2023)**. Vinpocetine was suspended in 0.5% sodium carboxymethyl cellulose (CMC). Pregabalin, dissolved in sterile saline, was administered at a dose of 10 mg/kg/day **(Yokoyama et al., 2007)**. Vinpocetin and pregabalin were administered to the respective groups orally, once daily for 4 weeks. Whereas the control group received the respective vehicles for the same duration.

2.3. Behavioral Tests

The study evaluated the long-term reflexive (antinociceptive), and non-reflexive (anxiolytic and antidepressant) pain responses. The antinociceptive effect was evaluated using pin-prick test, von Frey filament and tail-flick test. The anxiolytic response was evaluated using the elevated plus maze test. The antidepressant effect was ascertained by two tests, forced swimming tests and sucrose preference, in order to boost the integrity of the findings.

The behavioral assessment was performed at the 5th week (28 days after the last ASI injection) by a person who was blinded to the injection solution at scheduled times (9 a.m. to 2 p.m.). Every attempt has been made to minimize animal suffering and to ensure consistent results.

2.3.1. Pin prick test

To evaluate mechanical hyperalgesia, a modified pin prick test was employed wherein the plantar surface of the ipsilateral hind paw was precisely pressed by a safety pin without penetrating the skin **(Kaur and Muthuraman, 2019)**. Results were recorded according to the following scale "0=no response; 1=rapid paw flicking, stamping, or shaking (less than 1 s); 2=repeated paw stamping, shaking, or paw lift less than 3 s; 3=above behaviors or hind paw licking for more than 3 s; 4=above behaviors for more than 3 s". The average response for 3 trials was recorded **(Coderre et al., 2004)**.

2.3.2. von Frey filament test

To evaluate mechanical allodynia, mice were positioned on an elevated mesh platform for about 10 minutes prior to the commencement of the test to adapt the test chambers. Then, the plantar surface of the right hind paw was stimulated ten times by 35.6 mN von Frey filament pressure through the mesh at intervals of 3–4 sec. A positive withdrawal response was recorded if an evident withdrawal, shaking, licking, or lifting the paw occurred upon application of the filament. The percentage of withdrawal frequency was assessed as follow: "number of paw withdrawals/number of trials × 100" **(Deuis et al., 2017)**.

2.3.3. Tail-flick warm water test

Thermal hyperalgesia was assessed using the tail-

flick test where the tail of each animal was submerged in a water bath that was kept at 50 ± 0.5 °C. The latency to abrupt retraction of the tail was noted. The time should not exceed 10 seconds so as to avert tissue damage **(Deuis et al., 2017)**.

2.3.4. Elevated plus maze test

To ascertain anxiety behavior, each animal was placed at the center of the maze, oriented toward an open arm and permitted to move freely for few minutes, then returned to its home cage. The percentage of open arm entries "number of entries into the open arms/number of open + closed arm entries * 100", and the percentage of time spent in the open arms "% time = the duration spent in the open arm /the total duration in the open and closed arms * 100" were calculated **(Rodgers and Dalvi, 1997; Liu et al., 2017)**.

2.3.5. Forced swimming test (FST)

Mice were individually forced to swim in a 40×15 cm plastic cylinder containing plain water, up to 35 cm height, for 5 minutes. The animal was considered immobile when it did not do any activity rather than that required to keep its head above water level. An increased duration of immobility reflects a state of despair **(Toledano-Martos et al., 2024)**. As the FST is a stressful task, it was conducted after the other behavioral measures to minimize potential influence.

2.3.6. Sucrose preference test (SPT)

The SPT is a reliable index for evaluating anhedonia, a fundamental symptom of depression in rodents **(Markov, 2022)**. Mice were allowed to freely drink from 2 identical bottles, with their locations being switched around, containing either plain water or 20% sucrose solution prepared by dissolving 20 grams of sucrose in 100 ml water **(Liu et al., 2014b)**. The total amount of consumed solution was determined to calculate percentage of sucrose consumption to total fluid consumption **(Yu et al., 2019)**.

2.4. Biochemical estimation

After performing the behavioral tests, mice were fasted for the whole night, then they were deeply anesthetized with i.p. ketamine (80 mg/kg) **(Navarro et al., 2021)**. Blood samples were immediately drawn from the abdominal aorta and centrifuged for 15 minutes. The brain and spinal cord were precisely dissected, rinsed with phosphate-buffered saline. Then, the hippocampus was cautiously separated, and specimens were rapidly frozen at -80 for biochemical analysis.

The level of thiobarbituric acid reactive substances (TBARS) was determined in serum and tissue (brain and spinal cord) supernatants using the provided kit (Bio-diagnostic, Cairo, Egypt) following previous method **(Tappel and Zalkin, 1960)**. After dilution with PBS, samples were incubated for 2 hours with butylhydroxytoluene and trichloroacetic acid then centrifuged for 15 minutes. The optical density of resultant pink color adducts following the reaction with 2-thiobarbituric acid was read at 532 nm.

Levels of antioxidants reduced glutathione (GSH) and superoxide dismutase (SOD) in the serum, brain and spinal cord were estimated according to the manufacturer instructions (Bio-diagnostic, Cairo, Egypt). For GSH, samples had been thoroughly mixed with DTNB. The absorbance of the colored product can be read at 412 nm, and GSH level was expressed as µmol/mg protein. For SOD, pyrogallol has been used as substrate where the amount of SOD (U/mg protein) that inhibits the rate of pyrogallol auto-oxidation by 50% reflects SOD activity **(Ellman, 1959; Marklund and Marklund, 1974)**.

The levels of IL-1β. IL-18, TNF-α and iNOS were determined in serum (ng/dl), and the homogenate supernatant from brain and spinal cord (ng/µg protein) following the provided manufacturer's guidance with Sandwich ELISA Kit (INOVA, Beijing, China). The absorbance was measured at 450 nm (ELISA reader, ELx800, Bio-TEK) **(Tian et al., 2018; Yoshida et al., 2018)**.

2.4.1. Western blot

Tissue samples of hippocampi were homogenized on ice in RIP assay buffer containing 10 mM Tris-HCl (pH 7.4), 0.1 % sodium dodecyl sulfate, 150 mM NaCl, 1 % Triton X-100, 1 mM EDTA, NaF and phenylmethylsulphonyl fluoride (PMSF). Then, samples were centrifuged (10,000 rpm) for 30 min at to separate the supernatant. The protein concentration was determined, and samples were separated on 12 % SDS-PAGE and prepared for incubation with primary antibodies overnight at 4°C. The primary antibodies ERK1/2 (4695), pERK1/2 (4370) were obtained from Cell signaling technology (Beverly, MA, USA). For incubation with anti-rabbit secondary antibody (7054), the

membrane was rinsed in Tris-buffered saline with Tween-20 (TBST), then washed and subjected to NBT/BCIP solution to observe the protein bands. Azure Imaging System 600 (Biosystems, USA) was used, and the intensity of each band was adjusted against β-actin (4970) levels.

2.5. Statistical Analysis

After testing the normality using Shapiro and D'agstino tests, parametric data had been compared by one way ANOVA followed by Tukey's *post hoc* test. Results are expressed as mean ± standard deviation (SD). When $p < 0.05$, results were deemed to be statistically significant. All data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp).

3. Results

3.1. Behavioral Tests

3.1.1. Effect of vinpocetine and pregabalin on reflexive pain responses

Administration of vinpocetine was beneficial in attenuating the mechanical allodynia in the right hind paw as evidenced by a significant increase in latency to mechanical stimulation when it was assessed 4 weeks after acidic saline injection ($p < 0.05$ compared to ASI group, **Table 1**). Similarly, pregabalin decreased allodynia compared to ASI group ($p < 0.05$), with no noticeable difference between vinpocetine and pregabalin treatment ($p > 0.05$).

Likewise, vinpocetine treatment exerted a significant reduction in mechanical hyperalgesia when compared to vehicle administration following injection of acidic saline ($p < 0.05$ compared to ASI group, **Table 1**). Additionally, there was no significant difference between the analgesic response of vinpocetine and pregabalin groups ($p > 0.05$ between vinpocetine and pregabalin treated groups).

On the other hand, thermal stimulation was associated with a non-significant group differences in tail withdrawal latencies reflecting that acidic saline model of FM did not maintain thermal hyperalgesia at 4 weeks after the last acidic saline injection **(Table 1)**.

3.2. Effect of vinpocetine and pregabalin on anxiety-like behavior

The evident reduction in the percentage of entries, and the time spent into the open arms of the maze compared to the normal control group indicates a state of anxiety in the ASI group ($p < 0.001$, **Table 1**). Vinpocetine mitigated the symptoms of anxiety as displayed by the significant increase in the frequency and duration spent in the open arms where it approximately reached the normal control value ($p < 0.01$ compared to ASI group, $p > 0.05$ compared to normal control group). Pregabalin only relieved the duration spent in the open area ($p < 0.05$ compared to the ASI group), nevertheless, it remained significantly lower than the control group ($p < 0.05$ compared to the normal control group).

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3.1.3. Effect of vinpocetine and pregabalin on depression-like behavior

FST and SPT are robust indices of despair and anhedonia, respectively, the core symptoms of depression.

3.1.4. The forced swimming test

Our results depicted a significant improvement in the immobility time in Vinpo + ASI group relative to ASI group ($p < 0.01$, **Table 1**). In contrast, the duration of immobility in pregabalin treated group could not be significantly distinguished from that of the ASI group ($p > 0.05$).

3.1.5. Sucrose preference test

The ASI group showed a significant decrease in the preference of sucrose compared to normal control reflecting an anhedonic behavior ($p < 0.05$, **Table 1**). In line with FST, vinpocetine reversed the

Table 1. **Effect of vinpocetine and pregabalin on reflexive and non-reflexive pain responses in acidified saline-induced fibromyalgia model**

	Control	ASI	ASI+Vinpo	ASI+Preg
Pin prick test				
Nociceptive score	0.22±0.27	3.44^{a} ±0.46	1.38^{b} ±0.38	1.22^{b} ±0.40
von Frey test				
Withdrawal frequency (%)	15±8.3	75^{a} ±12.24	38.33^{b}±11.69	35^{b} ±10.4
Tail flick test				
Latency time	7.5±0.54	6.83 ±0.98	7.16±0.98	7.0±0.89
Elevated plus maze test				
% time spent in the open arm	43.37 ± 3.78	10.23^{a} ± 2.26	38.05^{b} ± 6.72	25.77^{abc} ± 25.77
% open arm entries	44.33 ± 3.14	25.77^{a} ± 5.57	38.40^{b} ± 7.43	29.75^{a} ± 6.78
FST				
Immobility time (seconds)	18.50 ± 6.35	47.50^{a} ± 13.72	29.67^{b} ± 8.14	32.17^{a} ± 8.75
SPT				
% Sucrose preference	73.83 ± 9.02	35.50^{a} ± 4.93	60.67^{b} ± 10.39	40.33^{ac} ± 8.87

ASI; acidified saline solution-treated group, ASI+Vinpo; ASI and vinpocetine-treated group, ASI+Preg; ASI and pregabalin-treated group. Data are represented as the mean of ten animals in each group. [a] $p < 0.05$ compared to control group; [b] $p < 0.05$ compared to ASI group; [c] $p < 0.05$ compared to ASI + Vinpo group

anhedonic response relative to ASI group whereas pregabalin non-significantly affect such response ($p > 0.05$ compared to ASI group).

3.2. Biochemical estimations

3.2.1. Effect of vinpocetine and pregabalin on oxidative stress (OS) parameters

The results showed a significant elevation in the level of thiobarbituric acid (TBA) reactive substances observed in the serum as well as in spinal cord and brain homogenates obtained from ASI group compared to control group ($p<0.001$, **Fig. 1**). Both vinpocetine and pregabalin significantly decreased TBARS compared to ASI group ($p<0.01$). Note that although vinpocetine reduced brain TBARS compared to ASI group, it did not approach control values ($p<0.05$ compared to control, **Fig. 1**).

Serum GSH level remained unchanged ($p>0.05$) although it was significantly attenuated in spinal cord and brain samples from the ASI group compared to control ($p<0.05$, **Fig. 2**). Treatment with vinpocetine or pregabalin mitigated the reduction in GSH level in the spinal cord and brain tissues as depicted by its significant increase compared to ASI group ($p<0.001$, **Fig. 2**). It seems however that vinpocetine was more efficient in raising GSH levels in the spinal cord and brain as illustrated by the significant change between the

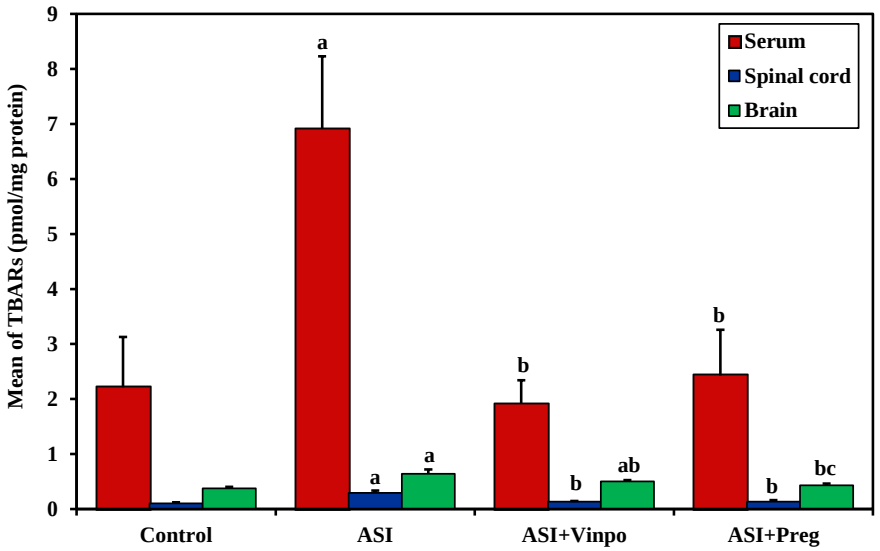

Fig. 1. Effect of vinpocetine and pregabalin on TBARs in serum, spinal cord, and brain homogenates. ASI; acidified saline solution-treated group, ASI+Vinpo; ASI and vinpocetine-treated group, ASI+Preg; ASI and pregabalin-treated group; TBARs; thiobarbituric acid reactive substances. Data are represented as the mean of ten animals in each group. [a] $p < 0.05$ compared to control group; [b] $p < 0.05$ compared to ASI group; [c] $p < 0.05$ compared to ASI + Vinpo group.

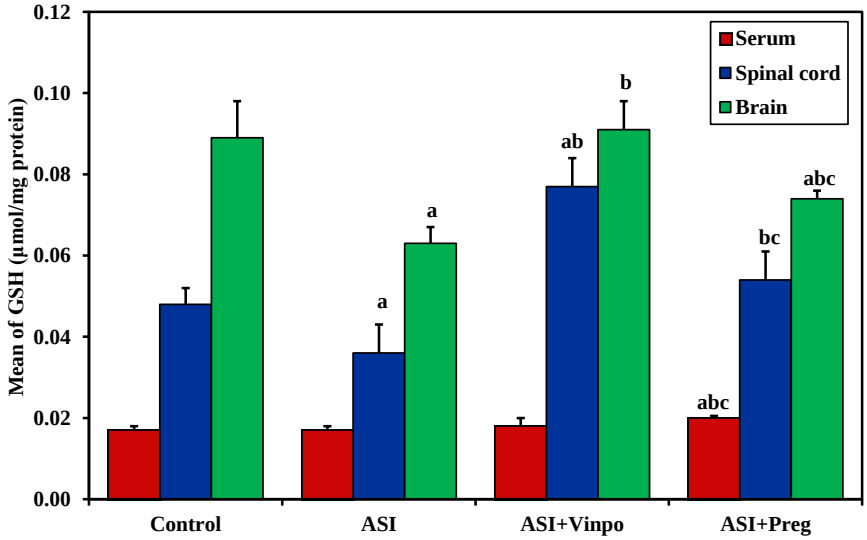

Fig. 2. Effect of vinpocetine and pregabalin on GSH in serum, spinal cord, and brain homogenates. ASI; acidified saline solution-treated group, ASI+Vinpo; ASI and vinpocetine-treated group, ASI+Preg; ASI and pregabalin-treated group; GHS; glutathione. Data are represented as the mean of ten animals in each group. [a] $p < 0.05$ compared to control group; [b] $p < 0.05$ compared to ASI group; [c] $p < 0.05$ compared to ASI + Vinpo group.

vinpocetine and pregabalin treated groups (p<0.05, **Fig. 2**).

The reduction in the antioxidant enzyme, SOD was evident in the serum as well as tissue homogenates from the ASI group compared to the control (p<0.001, **Fig. 3**). Both vinpocetine and pregabalin treatment significantly restored SOD (p<0.05 compared to ASI group). In line with GSH results, SOD was significantly increased in the spinal cord and brain from vinpocetine group relative to pregabalin group (p<0.01, **Fig. 3**).

3.2.2. Effect of vinpocetine and pregabalin on inflammatory markers

Injection of acidic saline resulted into a significant increase in IL-1 level in the serum and brain homogenates from ASI control group compared to normal control values (p<0.001, **Fig. 4 A&B**). Vinpocetine and pregabalin significantly reduced IL-1 levels in the serum and brain compared to ASI group (p<0.001). Strikingly, IL-1 levels in spinal cord homogenates did not significantly differ among the four groups (p = 0.066, **Fig. 4C**).

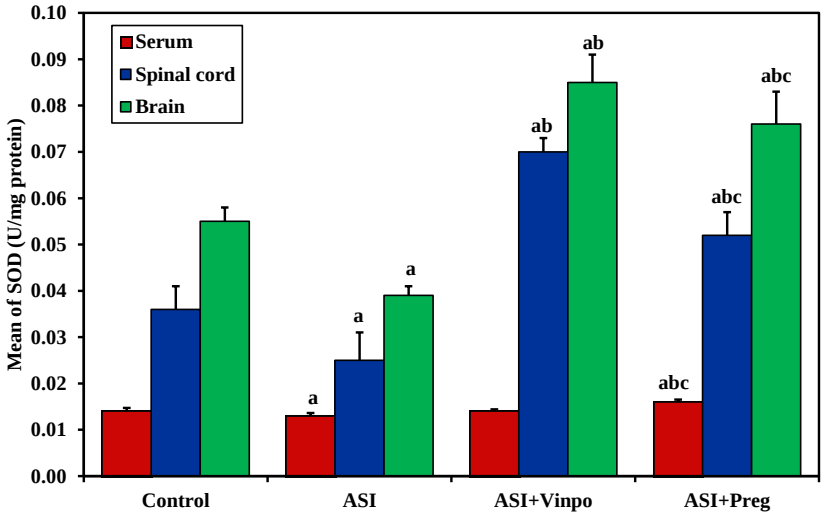

Fig. 3. Effect of vinpocetine and pregabalin on SOD in serum, spinal cord, and brain homogenates. ASI; acidified saline solution-treated group, ASI+Vinpo; ASI and vinpocetine-treated group, ASI+Preg; ASI and pregabalin-treated group; SOD; superoxide mutase. Data are represented as the mean of ten animals in each group. [a] $p < 0.05$ compared to control group; [b] $p < 0.05$ compared to ASI group; [c] $p < 0.05$ compared to ASI + Vinpo group.

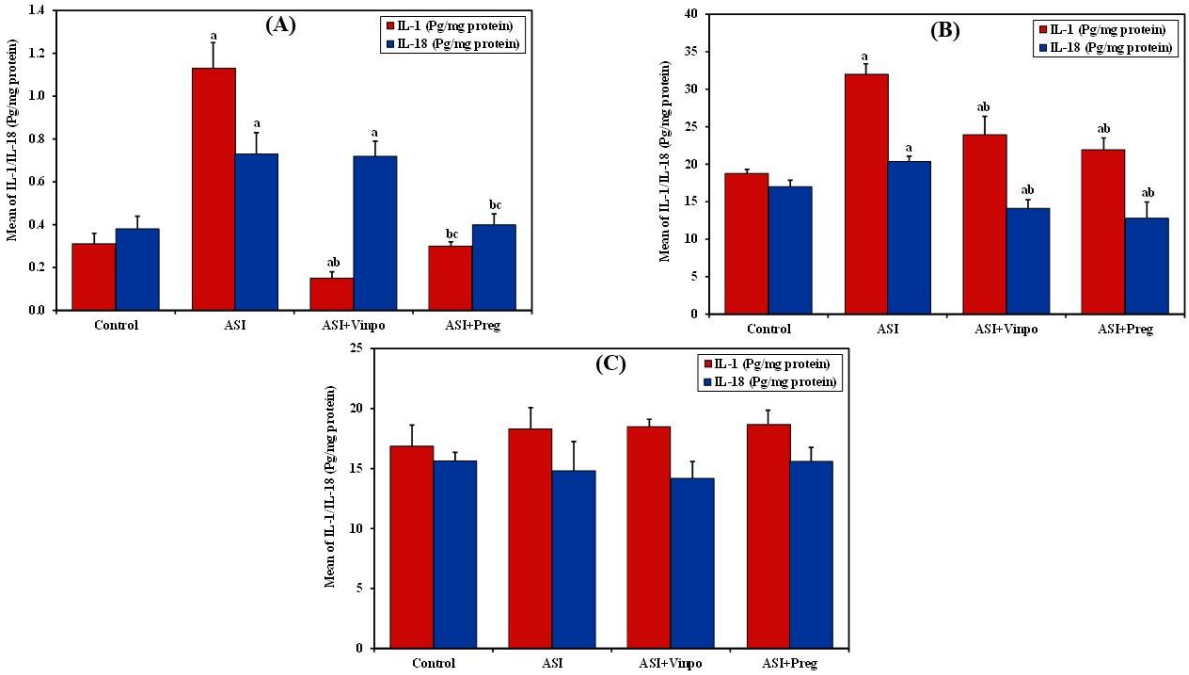

Fig. 4. Effect of vinpocetine and pregabalin on IL1, IL-18 in the serum (A), brain (B), and spinal cord (C). ASI; acidified saline solution-treated group, ASI+Vinpo; ASI and vinpocetine-treated group, ASI+Preg; ASI and pregabalin-treated group; IL-1; interleukin1, IL-18; interleukin-18. Data are represented as the mean of ten animals in each group. [a] $p < 0.05$ compared to control group; [b] $p < 0.05$ compared to ASI group; [c] $p < 0.05$ compared to ASI + Vinpo group.

Likewise, IL-18 levels did not elevate in the spinal cord homogenates obtained from ASI group (p=0.2, **Fig. 4C**) although it significantly increased in the serum and brain tissue homogenates from ASI group compared to control group (p<0.001, **Fig. 4A&B**). Vinpocetine significantly reduced IL-18 in the brain (p<0.001 compared to ASI group, **Fig. 4B**), while pregabalin decreased IL-18 in the serum and brain homogenate (p<0.001 compared to ASI group, **Fig. 4A&B**).

As depicted from **Fig. 5**, TNF-α was significantly elevated in the serum as well as spinal cord and brain obtained from ASI control group. Both vinpocetine and pregabalin significantly reduced TNF-α in the serum and spinal cord compared to ASI control group (p<0.001), with no significant change from control values (p>0.05). In the brain, TNF-α was also significantly lowered by vinpocetine and pregabalin treatment compared to ASI group (p<0.001), while it remained higher than control values (p<0.05).

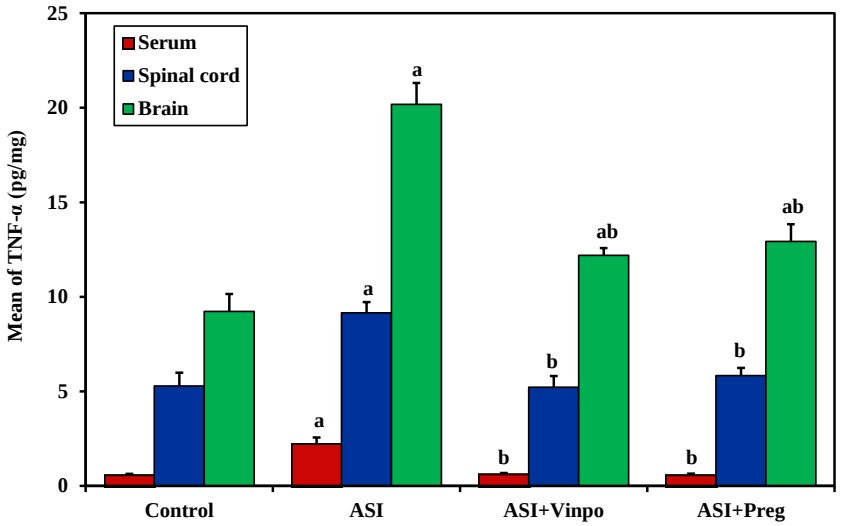

Fig. 5. Effect of vinpocetine and pregabalin on TNF-α in serum, spinal cord, and brain homogenates. ASI; acidified saline solution-treated group, ASI+Vinpo; ASI and vinpocetine-treated group, ASI+Preg; ASI and pregabalin-treated group; TNF-α; tumour necrosis factor alpha. Data are represented as the mean of ten animals in each group. [a] $p < 0.05$ compared to control group; [b] $p < 0.05$ compared to ASI group.

3.2.3. Effect of vinpocetine and pregabalin on iNOS

Results revealed a significant increase in iNOS level in the serum as well as spinal cord and brain tissues obtained from ASI control group. Both vinpocetine and pregabalin significantly reversed the elevation in iNOS levels where there was a significant difference between vinpocetine or pregabalin groups compared to ASI group (p<0.001, **Fig. 6**).

3.2.4. Effect of vinpocetine and pregabalin on ERK expression

Our results revealed a significant reduction in the expression of p-ERK in the hippocampi from ASI control group. On the other hand, vinpocetine significantly improved p-ERK expression compared to ASI control group and pregabalin treated group (p<0.01, **Fig. 7**).

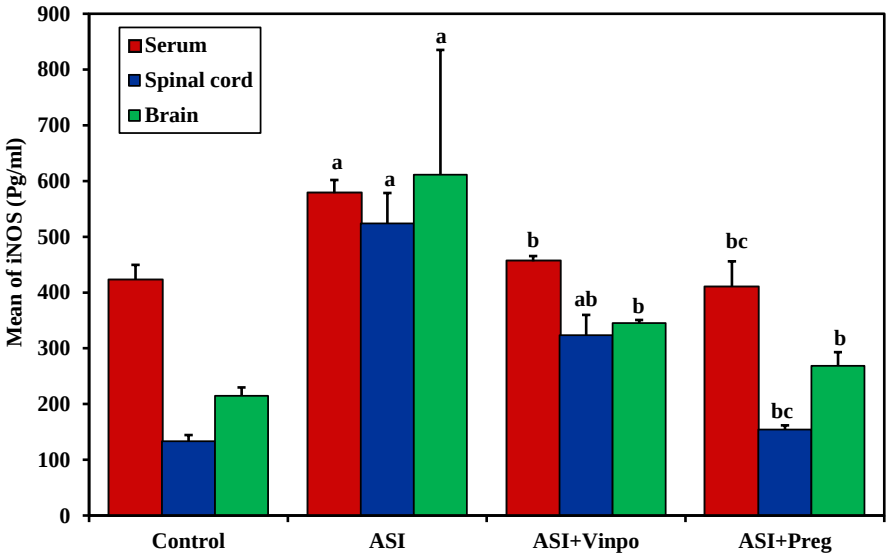

Fig. 6. Effect of vinpocetine and pregabalin on iNOS in serum, spinal cord, and brain homogenates. ASI; acidified saline solution-treated group, ASI+Vinpo; ASI and vinpocetine-treated group, ASI+Preg; ASI and pregabalin-treated group; iNOS; inducible nitric oxide synthase. Data are represented as the mean of ten animals in each group. [a] $p < 0.05$ compared to control group; [b] $p < 0.05$ compared to ASI group; [c] $p < 0.05$ compared to ASI + Vinpo group.

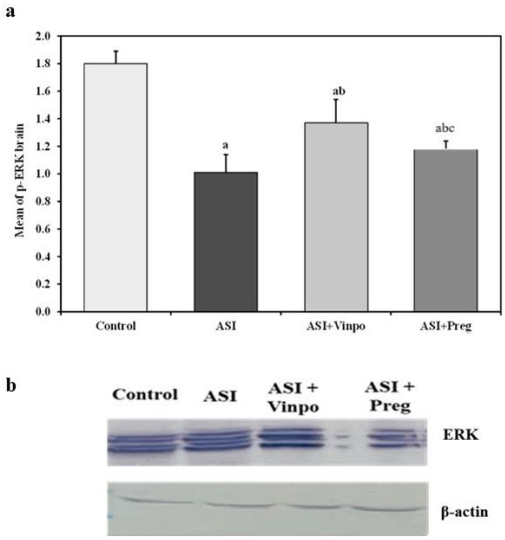

Fig. 7. Effect of vinpocetine and pregabalin on ERK expression in hippocampi homogenates. (a) The level of nuclear protein expression of ERK was normalized to that of β-actin. (b) Representative immunoblots for ERK and β-actin proteins. ASI; acidified saline solution-treated group, ASI+Vinpo; ASI and vinpocetine-treated group, ASI+Preg; ASI and pregabalin-treated group; iNOS; inducible nitric oxide synthase. Data are represented as the mean ± SD (n = 10 per group) and analyzed by one-way ANOVA followed, when significant, by followed by Post Hoc test. [a] $p < 0.05$ compared to control group; [b] $p < 0.05$ compared to ASI group; [c] $p < 0.05$ compared to ASI + Vinpo group.

4. Discussion

Repeated intramuscular injections of acidic saline in the gastrocnemius muscle have been shown to mimic a model of chronic widespread pain (CWP) syndromes in experimental animals **(Sluka and Clauw, 2016)**. Data revealed that the acid saline model shows behavioral deficits that develop late and last longer, even in the absence of reflexive pain responses **(Álvarez-Pérez et al., 2022)**.

Our study adds to the evidence validating this model of FM-like symptoms where a significant hypersensitivity to innocuous mechanical stimulation coupled with depressive symptoms had been developed. Activation of acid sensing (AS) ion channels, particularly ASIC3, mediates aberrant pain response as evidenced by the abolishment of the development of hyperalgesia upon pharmacological blockade of ASIC by its selective blocker **(Gregory et al., 2016; Yen et al., 2017)**. Nevertheless, the use of ASIC blocker after the establishment of hyperalgesia has no obvious effect, reflecting lack of involvement in the maintenance of hyperalgesia following repeated muscle insults **(Gautam et al., 2012)**.

Previous studies have demonstrated the role of ILs as critical participants in neurogenic inflammation **(Clark et al., 2006; Sutton and Opp, 2015; Littlejohn and Guymer, 2018)**. Of concern, inflammatory signals play a crucial role in the generation and enhancement of FM symptoms and related comorbidities **(Üçeyler et al., 2011; O'Mahony et al., 2021)**. Both IL-1 and IL-18 have a crucial role in several biological responses associated with neuroinflammation **(Kaplanski, 2018)**. Moreover, IL-18 has also emerged as a conceivable therapeutic target in various neurodegenerative disorders and chronic pain **(Tapia et al., 2019; Ju et al., 2024)**. Within the spinal cord, IL-18 released from microglia activates its receptor expressed on astrocytes, triggering a pain response by activating a cascade of different molecules. This could explain the connection between microglia and astrocytes activation in chronic pain conditions **(Miyoshi et al., 2008)**.

Pilat et al. (2016) demonstrated an elevation of the expression of IL-18 and its receptor in the spinal cord up to 14 days in a model of neuropathic pain. **Yoshida et al. (2018)** demonstrated that IL-18 have been upregulated in a model of muscle pain induced by repetitive electric stimulation. Furthermore, antagonizing IL-18 reduced hyperalgesia in stimulated muscles. Herein, we observed a significant increase in IL-1 and IL-18 in the brain homogenate and serum. However, there was a nonsignificant increase in their levels in spinal cord homogenates of ASI groups. As we measure the interleukins levels at one time point, we can suggest that their levels could be formerly changed. Hence, further studies are needed to measure ILs at different time points in this FM

model.

The current study demonstrated also an evident increase of the markers of oxidative stress in conjunction to inflammatory reaction both centrally and peripherally. These results are in line with the critical role of OS in the development of nociplastic pain **(Yüksel et al., 2017; Brum et al., 2022)**.

Additionally, our results showed that both vinpocetine and pregabalin significantly improved reflexive pain response in FM rats, in conjunction with amelioration of inflammatory and OS markers. On the other hand, vinpocetine more significantly improved anxiety and depressive like behavior compared to pregabalin.

Vinpocetine has long been known for its protective effects in cerebrovascular incidents via restoring cerebral flow and energy balance **(Lourenco-Gonzalez et al., 2019)**. In addition, mounting data have documented that vinpocetine, via modulating glutamate release and Na+ conductivity, exerts a beneficial role in learning and memory deficits **(Filgueiras et al., 2010; Zhang et al., 2018)**, and alleviate cognitive performance and depressive illness in patients suffering from organic psychosyndromes **(Hindmarch I et al., 1991)**.

Multiple mechanistic actions underpin the neuroprotective effects of vinpocetine. It modulates voltage-dependent ion channels in neuronal cells and enhances acetylcholinesterase activity **(Bonoczk et al., 2000)** and increased the brain level of antioxidant enzymes **(Ishola et al., 2018)**.

Vinpocetine has also been shown to exert a potent anti-inflammatory action that has been reported in various organs including heart **(Abdelmageed et al., 2023)**, kidneys **(Fattori et al., 2017)**, retina **(Liu et al., 2014a)**, and urinary bladder **(Abdelrahman et al., 2023)**. The distinct anti-inflammatory action of vinpocetine has been attributed to its direct effect on IKK and subsequent inhibition of NF-κB and its downstream proinflammatory cytokines **(Jeon et al., 2010)**. Moreover, vinpocetine has been shown to modulate Akt phosphorylation, the upstream regulator of IKK **(Lourenco-Gonzalez et al., 2019)**. Vinpocetine has been shown to upregulate BDNF-related proteins and downregulate GSK-3β/β-catenin pathway. **(Ahmed et al., 2018; Xu et al., 2019)**. Furthermore, **Han et al. (2020)** showed that vinpocetine represents a neuroprotective effect in a model of ischemic stroke by regulating inflammation via targeting NLRP3 inflammasome signaling pathway.

Based on its multiple mechanisms of action, previous research hypothesized vinpocetine as a promising agent in different models of pain. **Ruiz-Miyazawa et al. (2015)** demonstrated that vinpocetine reduced hyperalgesia when it was given 1 h before LPS injection. More recently, **Lourenco-Gonzalez et al. (2019)** found that vinpocetine reduced pain and restored oxidative/antioxidative balance in a model of KO2-induced pain. Additionally, **Colombo et al. (2018)** documented for the first time an analgesic effect of vinpocetine in a model of experimental colitis in mice. In line, we observed favorable effects of vinpocetine on mechanical allodynia and hyperalgesia and depressive like behaviors in this model of FM in conjunction with restoration of OS balance, and amelioration of ILs, TNF- α and iNOS tissue levels.

We also aimed to investigate the effect of vinpocetine on ERK expression in this FM model. We showed for the first time that vinpocetine enhanced ERK expression in line with amelioration of depressive behavior.

ERK and its downstream signaling molecules have an important contribution in cell proliferation and other physiological functions **(Lavoie et al., 2020)**. They are pivotal in promoting neuronal plasticity and modulating neurotransmitter release **(Marsden, 2013)**. Data shows that certain neurological disorders are associated with adaptive changes in ERK1/2 signaling pathways that regulate a network of genes associated with the development of behavioral and cognitive impairment **(Wang and Mao, 2019; Hu et al., 2023)**.

Herein, our results showed suppression of ERK expression in the brain of acidic saline-treated animals. This result runs in parallel to previous studies that showed decreased ERK expression in brain regions from depressed suicide patients, and animal models of chronic but not acute depression **(Dwivedi et al., 2001; Wang and Mao, 2019)**. This notion led researchers to investigate the effect of antidepressants on ERK signaling and the outcome of certain agents that enhance ERK expression on depressive symptoms **(Qi et al., 2008)**. Recently, Hu et al. showed that esketamine upregulated PGC-1α and irisin expression that in turn activate ERK1/2 and ameliorate depressive like symptoms in mice **(Hu et al., 2023)**.

The effect of ERK activation as a fundamental process mediating various biological functions is double edged in FM. On one hand, ERK exerts vital biological effects on nerve regeneration where suppression of ERK might be involved in cognitive impairment and behavioral changes **(Duman and Voleti, 2012)**. On the other hand, ERK activation could be linked to neuronal toxicity and enhanced excitability **(Zhen et al., 2023)**. Thus, it is imperative to approach drugs that modulate ERK pathway cautiously to mitigate the risk of central adverse effects manifested mainly as behavioral and mental impairment coinciding with decline in nerve regeneration. Vinpocetine can add value in FM management without suppressing ERK signaling. This effect could be attributed to its regulatory effect on the endogenous neurotrophic factors such as BDNF, an upstream regulator of ERK1/2 **(Ahmed et al., 2018; Xu et al., 2019)**.

6. Conclusion

In conclusion, our study demonstrated that vinpocetine could be a promising agent in the management of FM. The observed improvement in the behavioral alterations may be attributed to attenuation of neuroinflammation and oxidative stress together with upregulation of ERK expression.

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Authors' contributions

W. A. conceived and designed the research, carried out the experiments, and analyzed data. D.G. and A.M. performed the biochemical analysis and Western blotting. W.A. wrote the original draft of the manuscript. All authors reviewed the final version of the manuscript.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the Faculty of Medicine, Alexandria University, Alexandria, Egypt (IRB No: 00012098-serial No: 0306041).

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