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Jobelyn® Ameliorates Anxiety-like Behaviour, Thermal Hyperalgesia and Neuroinflammation in Formaldehyde-Induced Arthritis in Mice

Adaeze N. Adebesin[1], Gbemisayo A. Abbas[2,3], Anjolajesu M. Ogunsola[1], Oluwafolaranmi O. Mabekoje[1], Damilola J. Adebambo[1], Joy M. Oni[1], Habeeb A. Adeogun[1], Opemipo Akinyemi[1], Itunu R. Ayoade[1], Adetoke M. Ifesanwo[1], Abayomi M. Ajayi[3]

[1]Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Sagamu Campus, Nigeria.
[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Lead City University, Ibadan, Nigeria.
[3]Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Nigeria.

ABSTRACT

Arthritis describes a chronic inflammatory disease characterised by joint pain, stiffness, and swelling. Anxiety and depression are common comorbidities in rheumatoid arthritis, a major type of common arthritis in patients, which can worsen disease outcomes. Many medicinal herbs with various sites of action against inflammatory and oxidative processes that play important roles in arthritis pathogenesis are being studied as potential novel treatments. Jobelyn® (JB), a polyphenol-rich extract, has been shown to relieve arthritic pain and possess anti-inflammatory and antioxidant properties. This study investigated the effects of JB on anxiety-like behaviour, thermal hyperalgesia, and neuroinflammation in a mouse model of formaldehyde-induced arthritis. Thirty Swiss male mice were grouped into 5 (n=6) as follows: control (distilled water), formaldehyde-induced arthritis group (FIA only) (2.5%), FIA + JB 50mg/kg, FIA + JB 100mg/kg, FIA + JB 200mg/kg and FIA + celecoxib 20mg/kg for 7 days. Anxiety-like behaviour was assessed using an elevated plus maze (EPM) and an open field test (OFT). Thermal hyperalgesia was evaluated using the hot plate test. Oxidative stress and pro-inflammatory cytokines were assessed in brain and paw tissues. JB treatment significantly reduced anxiety-like behaviour in EPM and OFT, and thermal hyperalgesia on a hot plate in arthritic mice. JB decreased brain and paw tissue levels of malondialdehyde and nitrite and increased reduced glutathione, catalase, superoxide dismutase and glutathione-s-transferase. Furthermore, JB caused significant reduction in brain tumour necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) as well as paw TNF-α. This study demonstrates the potential of JB to ameliorate anxiety-like behaviour, thermal hyperalgesia, and neuroinflammation.

Keywords

Jobelyn®, Arthritis, hyperalgesia, Anxiety, Neuroinflammation

Correspondence: Adaeze N. Adebesin, PhD. Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Sagamu Campus, Nigeria. E-mail: adebesin.ngozi@oouagoiwoye.edu.ng; Phone Number:+2348032408432; ORCID: 0000-0002-8361-6868

Gbemisayo A. Abbas - abbas.gbemisayo@lcu.edu.ng, 0009-0003-0351-3629; Anjolajesu M. Ogunsola - anjolajesuogunsola12@yahoo.com; Oluwafolaranmi O. Mabekoje - mabekojefola@gmail.com; Damilola J. Adebambo - adebambodamilola865@gmail.com;, Joy M. Oni - joyoluwapelumi22@gmail.com; Habeeb A. Adeogun - adeogunhabeeb478@gmail.com; Opemipo Akinyemi - opemipoakinyemi80@gmail.com; Itunu R. Ayoade - ayoadeitunu17@gmail.com; Adetoke M. Ifesanwo - adetokeblessing@gmail.com; Abayomi M. Ajayi - yomexj@yahoo.com, 0000-0001-6586-0421

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INTRODUCTION

Arthritis is a chronic condition characterised by joint inflammation and pain (Firestein, 2018). It causes pain, swelling, and stiffness. It affects millions of people worldwide, causing significant disability and morbidity (Scott *et al.*, 2010). There are several models that can be used to study arthritis in animals, and the formaldehyde-induced arthritis (FIA) is one such experimental animal model used to study arthritis, particularly rheumatoid arthritis (RA). RA

is an autoimmune inflammatory disease that causes joint pain, swelling, and damage. The disease usually affects the synovial joints, producing discomfort, deformity, and functional limitations, leading to significant morbidity and mortality (Guo *et al.*, 2018). It is a chronic joint disorder that is a significant cause of disability worldwide, affecting the elderly population with a higher severity in females than in males, and its prevalence in the general population ranges from 0.5% to 2% (Conforti *et al.*, 2021).

Formaldehyde has been used to induce arthritis in animals since it causes a prolonged inflammatory reaction with immune system changes. The FIA model replicates the acute arthritic reaction in humans and is employed in preclinical investigations due to its excellent validity. Formaldehyde induces arthritis by breaking down proteins at the site of injection, which produces an immune response against the degraded substances (Nair *et al.*, 2012; Manan *et al.,* 2022). Arthritic action of formaldehyde consists of two phases. In the initial phase, substance P is released, while in the late phase bradykinin, histamine, serotonin, and prostaglandin are released, which results in marked permeability and vasodilation. These mediators are responsible for hyperalgesia by stimulating nerve terminals and pain receptors. Hence, hypersensitivity is evoked at the injection site (Manan *et al.,* 2022).

The prevalence of mood disorders like anxiety and sadness is high in arthritic patients due to the chronic nature of the disease and the knowledge that it cannot be cured (Peterson *et al.*, 2019), leading to a variety of emotional responses, such as depression, worry, sadness, exhaustion, anger, and social disengagement. Depression and anxiety are common mental health issues associated with arthritis (VanDyke *et al.,* 2004; Isik *et al.*, 2007). Approximately 20%–40% of patients with arthritis have either depression, anxiety or a combination of both (Matcham *et al.,* 2013; Zhao *et al.,* 2020).

Although a number of biomolecular pathways have been proposed to explain the aetiology of arthritis, oxidative stress and inflammation caused by immune and inflammatory cells entering the joints have been recognised as the primary causes of the disease and its progression (Wang *et al.*, 2022). Oxidative stress is said to trigger and worsen inflammation in the joints, a hallmark of arthritis. Nitric oxide-mediated nitrergic stress contributes to neuroinflammation, and an increase in brain nitric oxide levels has been implicated in disruption of hippocampal neuronal functions, loss of memory, and other neurologic disorders (Fagundes *et al.,* 2015). However, the aetiology of arthritis remains unclear. Nonetheless, many contributing factors have been identified as core pathological features, including complex interactions between genetic and environmental factors, as well as the infiltration of inflammatory and immune cells into joint tissues, which leads to the production of inflammatory mediators and oxidative biomarkers (Volkov *et al.*, 2020).

The major goal of treatment is to reduce pain, decrease inflammation, and improve the quality of life of the patients (Nafiu *et al.,* 2024). Medications such as celecoxib (CEL) and diclofenac have been used to manage arthritic pain. Previous studies showed that CEL produces significant improvements in pain and inflammation, and these effects are maintained during treatment for up to 24 weeks in clinical trials. The current methods for managing arthritis and related pain have limited efficacy, and their clinical usefulness have been compromised by the occurrence of side effects, as they focus on symptom relief, as a cure remains elusive. The treatment objectives aim to enhance patients' quality of life and reduce pain episodes and inflammation (Sizova *et al.*, 2008). As a result, there is a need to look into alternate treatments for arthritis. Many medicinal herbs with various sites of action against inflammatory and oxidative processes that play important roles in arthritis pathogenesis are being studied as potential novel treatments.

Jobelyn® (JB) is a dietary supplement made from the polyphenol-rich leaf sheath of *Sorghum bicolor*, a plant known for its nutritional and therapeutic properties worldwide (Makanjuola *et al.*, 2017). *S. bicolor* (L.) Moench has been cultivated in northeastern Africa for over 5000 years. It is commonly known as millet. Its grains contain starch, fat, and protein, and they are sources of bioactive nutrients like vitamin B, fat-soluble vitamins, micro- and macronutrients, carotenoids, and polyphenols (Przybylska-Balcerek *et al.*, 2019). Sorghum grains possess health benefits, including antioxidant, anti-inflammatory and anticancer activities (Rao *et al.*, 2018). Pharmacological studies have also demonstrated that JB has antiamnesic, antidepressant, antioxidant, anti-inflammatory, and neuroprotective properties in rodents. These effects were linked to its high flavonoid-based polyphenolic phytochemicals such as luteolin, naringenin, apigenin, luteolidins, apigeninidins, and dimeric 3-deoxyanthocyanidin (Makanjuola *et al.*, 2017; Umukoro *et al.,* 2019). Previous research has indicated that JB has anti-inflammatory, antioxidant, and antidepressant activities (Benson *et al.*, 2013; Umukoro *et al.,* 2014). JB's high polyphenol content has also been connected to health benefits such as an immune booster (Omorogbe *et al.*, 2018; Adebesin *et al.,* 2024; Ajayi *et al.*, 2024). Its anti-arthritis effect in an immunological model of complete Freund adjuvant (CFA)-induced arthritis in Wistar rats have been previously reported (Omorogbe *et al.*, 2018; Abbas *et al.*, 2024). However, this study aimed to investigate the potential of JB to ameliorate anxiety-like behaviour, thermal hyperalgesia, and neuroinflammation in a non-immunologic model of FIA in mice.

MATERIALS AND METHODS

Experimental Animals

Thirty-six male Swiss mice (20–25 g) were used for this study. The mice were housed at room temperature (20-25°C) in plastic cages with a 12:12 natural light-dark cycle and were allowed free access to a commercial rodent pellet diet and water *ad libitum*. They were acclimatised for one week before the commencement of experiments, and all experimental procedures were carried out in strict compliance with the National Institutes of Health's (NIH) ethical guidelines for the care and use of laboratory animals (NIH, 2003).

Preparation of JB and CEL

CEL (Medico Remedies PVT) and JB (Health Forever Products, Lagos, Nigeria) were administered orally after being dissolved in distilled water prior to usage. The JB dosages used in the study were selected in light of findings from earlier research (Umukoro *et al.,* 2014). The CEL dosage of 20 mg/kg was chosen based on the body of existing literature (Anilkumar *et al.*, 2017).

Experimental Procedures

FIA

In this experiment, the mice were randomly distributed into 6 groups (n= 6). Arthritis was induced by injecting 2.5% formalin into the hind paw of the mice on day one alone before treatments, then the various treatments were administered daily for 7 days. Mice in group 1, which served as a control, were given distilled water (10 mL/kg, orally). Mice in groups 2-6 received injections of 20 µL of 2.5% formalin beneath the sole of the hind paw (subplantar) on day 1 prior to treatment. Group 2 served as the FIA control, which also received distilled water (10 mL/kg, orally). Groups 3–5 received JB (50, 100 and 200 mg/kg, orally), while group 6 received CEL (20 mg/kg, orally). The various treatments, except formaldehyde injection, were administered daily for 7 days. The duration of paw licking and frequency as an initial response by mice in groups 2-6 that received subplantar injections was determined at 0–5 min (neurogenic phase or first phase) and 15–30 min (inflammatory phase or second phase) after the injection of formaldehyde. On days 2 and 7, the arthritis score was done as follows: 0 = no swelling, 1 = mild swelling, 2 = swelling and erythema of digits, 3 = severe swelling and erythema, 4 = gross deformity and inability to use the limbs. The weights of the animals were also monitored using an electronic weighing balance on days 1, 2, and 7.

Thermal Hyperalgesia Test

The effect of JB on pain sensitivity to thermal hyperalgesia in FIA mice was assessed on day 7 using the Ugo Basile hot/cold plate according to the method described by Eddy and Leimbach (1953). The Ugo Basile hot/cold plate was set at 55±1°C. Latency to nociceptive response (jumping or paw licking) was measured. A cut-off period of 10 sec was set, and the mean percentage of maximum possible effect (% (MPE) was determined.

Test for Locomotor Activity in the Open-Field Test

This test was done using an open field chamber on day 7 post-treatment. Each mouse was placed in the centre of the box for 5 min, and the number of lines crossed was counted, which indicated the motor activity. A line crossing is typically counted and recorded when a mouse moves from one square to another. This helps to evaluate general locomotor activity (Crawley, 1985).

Test for Anxiety-like Behaviour using Elevated Plus Maze

The elevated plus maze was utilised on day 7 to assess the effect of FIA on anxiety-like behaviour, following the approach previously published by Pellow *et al.* (1985). In brief, each mouse was placed at the centre of the arms of the apparatus, and the duration and frequency of entries into open and closed arms were recorded for 5 min and used to evaluate anxiety-like behaviour.

Biochemical Assessment

The animals were sacrificed after anaesthesia using ketamine (75 mg/kg) and diazepam (2.5 mg/kg). The paws and brains were isolated, weighed and kept in a 10% w/v phosphate buffer (0.1 M, pH 7.4). The paw tissues or the whole brains of the mice were homogenised with the 10% w/v phosphate buffer, and their supernatants were stored at −20°C until use for the different biochemical assays.

Determination of Oxidative Stress Parameters

Biochemical tests were performed on paw and brain tissues to determine the effects of JB on oxidative stress parameters.

Malondialdehyde (MDA) levels were determined after tisTNFsue deproteinisation with trichloroacetic acid (TCA) and incubation with thiobarbituric acid. Absorbance was measured at 532 nm, and MDA levels were evaluated in the brain and paw tissues as described by Varshney and Kale (1990).

The Griess procedure (Green *et al.*, 1982) was used to measure the brain and paw tissue nitrite as a nitrergic stress marker. One hundred microlitres of Greiss reagent (a 1:1 solution of 1% sulfanilamide in 5% phosphoric acid and 0.1% N-1-naphthyl ethylenediamine dihydrochloride) was added to 100 µL of the supernatant, and absorbance was measured at 540 nm. The brain nitrite concentration was estimated from a standard curve obtained from sodium nitrite (0–100 uM).

The Ellman's procedure (Moron *et al.*, 1979) was used to detect reduced glutathione (GSH) in both the brain and paw tissue samples after TCA deproteinisation. Absorbance was measured at 412 nm against a blank reagent using a spectrophotometer. The concentrations of GSH in the brain tissues are expressed as micromoles per gram of tissue (µmol/g tissue).

The adrenaline auto-oxidation method described by Misra and Fridovich (1972) was used to determine the activity of superoxide dismutase (SOD) in the brain and paw tissue samples. An aliquot of 0.1 mL of the brain sample was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was started by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline to the mixture, which was quickly mixed by inversion. The reference cuvette contained 2.5 mL of buffer, 0.3 mL of adrenaline and 0.2 mL of water. The increase in absorbance at 480 nm was monitored and recorded at 0 sec and every 60 sec for 180 sec (Misra and Fridovich, 1972).

The method published by Goth (1991) was used to determine catalase (CAT) activity in both the brain and paw tissues and represented as µmoles of hydrogen peroxide decomposed per min per milligram of protein (Unit/mg protein). The methodology established by Habig and Jakoby. (1981) was utilised for the glutathione-S-transferase (GST) assay in both the brain and paw tissues. The absorbance

was measured at 405 nm for 5 min with a microplate reader (LT4500, UK). The protein content was calculated using Lowry *et al.* (1951) methodology.

Determination of Tumour Necrosis Factor-Alpha in the Brain and Paw Tissues

The concentrations of tumour necrosis factor-alpha (TNF-α) in brain and paw tissue supernatant were evaluated using BioLegend's ELISA MAXTM Deluxe kit, following manufacturer instructions. Absorbance was measured at 450 nm using a microplate reader, and concentrations were represented as pg/mL.

Determination of Interleukin-6 in the Paw Tissues

The concentrations of interleukin-6 (IL-6) in paw tissue supernatant were estimated using the ELISA MAXTM Deluxe kit (BioLegend,) according to the manufacturers' instructions. Absorbance was read at 450 nm using a microplate reader, and concentrations were expressed as pg/mL.

Determination of Acetylcholinesterase (AChE) Activity in Brain

The concentrations of Acetylcholinesterase (AChE) activity in the brain tissue supernatant were determined following the method described by Ho and Ellman (1969). Briefly, the AChE activity in the homogenate was measured by adding 2.6 ml of phosphate buffer (0.1 M, pH 7.4), 0.1 mL of 5,5'-dithio-bis-2-nitrobenzoic acid and 0.4 mL of the homogenate. Then, 0.1 mL of acetylcholine iodide solution was added to the reaction mixture. The absorbance was read at 412 nm using a spectrophotometer, and the change in absorbance was measured at two-minute intervals for a period of 10 min. AChE activity was expressed as micromoles per minute per milligram of tissue (µmol/min/mg tissue).

Statistical Analysis

The data obtained were analysed and expressed as the mean ± standard error of the mean (SEM) using the GraphPad Prism software version 8.4.2. Statistical analysis of data was done using one-way analysis of variance (ANOVA), followed by the Tukey post-hoc test. P-values less than 0.05 ($P < 0.05$) were considered statistically significant.

RESULTS

Effect of JB on the Immediate Nociceptive Response in FIA Mice

The anti-nociceptive effect of JB on FIA as measured by paw licking time is illustrated in Figure 1. In the early phase, there was a significant increase in paw licking time in the FIA group when compared to the control. The treatment with JB (50, 100 mg/kg) was able to minimize paw licking time in comparison to the FIA group. The CEL group also showed a similar decrease in the paw licking time. In Figure 1 (B), at the late phase, JB (50, 100, and 200 mg/kg) and CEL reduced paw licking duration significantly [$F_{5, 25} = 351.6$, $P < 0.0001$] in the second phase of the test compared to the FIA group, with no significant difference to the control. The JB treatment showed a dose-dependent reduction in paw licking time as compared to the FIA.

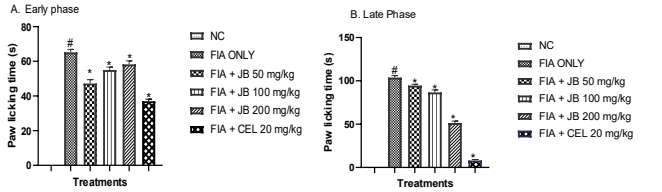

Fig 1: Anti-nociceptive activity of JB (50, 100 and 200 mg/kg) in the immediate phase of FIA: (A) First phase; (B) second phase. Values represent the mean ± SEM (n=5). #P < 0.05 indicates significance compared to the control group, while *P < 0.05 indicates significance compared to the FIA group, as determined by one-way ANOVA followed by Tukey's post hoc test.

Effect of JB on Arthritic Score and Thermal Hyperalgesia in FIA Mice

The inflammatory responses induced by subplantar injection of formaldehyde as measured by increase in arthritic scores are presented in Figure 2 (A). FIA increases arthritic scores when compared to the control, while treatment with JB (50, 100, 200 mg/kg) and CEL reduced the arthritic score in comparison to the FIA group, but there was no significant difference in the control groups. Figure 2 (B) shows the effects of JB on pain sensitivity in the hyperalgesia test in mice with subplantar injection of formaldehyde. The group that received FIA alone demonstrated a significant decrease in thermal latency compared to the control group. Oral doses of JB produced significant ($p < 0.05$) increases in thermal hyperalgesia latency in the hot plate test in comparison to the FIA-only group and control. The standard drug group, which was administered with CEL, also showed a similar increase to the JB-treated groups. The increase in hyperalgesia latency was consistent across all treatment groups and occurred over a longer time frame compared to the FIA group, which consistently showed low latency.

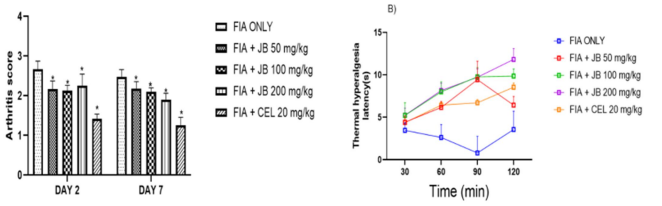

Fig. 2: Shows the effect of JB on (A) arthritic scores and (B) the thermal hyperalgesia test of FIA mice. Values represent the mean ± SEM (n=5). # p < 0.05 vs control, * p < 0.05 vs FIA using one-way ANOVA followed by Tukey's post hoc test.

Effect of JB on Locomotor Activity in the Open Field Test and Anxiety-Activity in Elevated Plus Maze

Figure 3 (A) shows the effect of JB on locomotor activity in the open field test and anxiety-activity in elevated plus maze. There was a significant effect ($p < 0.05$) on locomotor activity in treated mice JB (50, 100, 200 mg/kg) and CEL, as shown by the increased number of line crossings when compared to the FIA group. The untreated FIA group showed a reduction in the number of lines crossed, indicat-

ing decreased locomotor activity compared to the control group. The treatment groups, JB and CEL, increased line crossings compared to the FIA group, although the JB 50 mg/kg did not show a significant increase.

The effect of JB on anxiety-like activity in subplantar FIA mice is presented in Figures 3B and 3C. One-way ANOVA revealed that there were significant differences between treatment groups in the number of open arm entries [F 6, 26 = 41.71, p < 0.0001] and number of closed arm entries [F 6, 26 = 13.68, p < 0.0001] when compared with the FIA group. Groups administered with FIA alone showed a significant decrease in the number of open arm entries in the elevated plus maze paradigm when compared with control that wasn't exposed to formaldehyde. The various treatment groups significantly increased the number of open arm entries when compared with FIA administration. Also, the number of closed arm entry was significantly increased by FIA only when compared with control while this parameter was decreased significantly by the treatment groups with FIA when compared with FIA only group. JB (50, 100 and 200 mg/kg) or CEL (20 mg/kg) significantly (p < 0.05) attenuated the anxiety-like behaviour induced by FIA in mice.

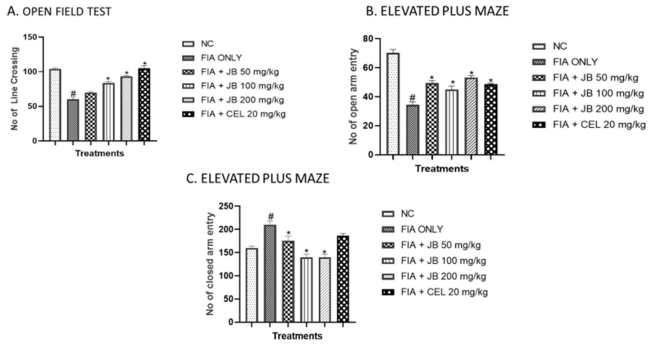

Fig. 3: Effect of JB on locomotor activity in the open field test: (A) number of line crossings and anxiety-like behaviours; (B) number of open arm entries, and (C) number of closed arm entries in FIA mice. Values represent the mean ± SEM (n=5). # P < 0.05 indicates significance compared to the control group, while *P < 0.05 indicates significance compared to the FIA group, as determined by one-way ANOVA followed by Turkey's *post hoc* test.

The Effect of JB on MDA and Nitrite Levels in the Brain and Paw Tissues of FIA Mice

This result indicated that the FIA-only group significantly increased the levels of MDA in both the brain and paw samples when compared with the control group that wasn't exposed to FIA at all. JB treatment and CEL significantly (p < 0.05) reduced FIA-induced elevation of MDA, an index of lipid peroxidation in brain and paw tissue (Fig. 4A and B) when compared to the FIA group only. Furthermore, the levels of nitrites in brain and paw tissue samples were elevated in the FIA group as compared with the control, while this elevation in brain and paw tissue nitrite levels was significantly reduced when compared to the FIA group only, as shown in Figures 5 C&D.

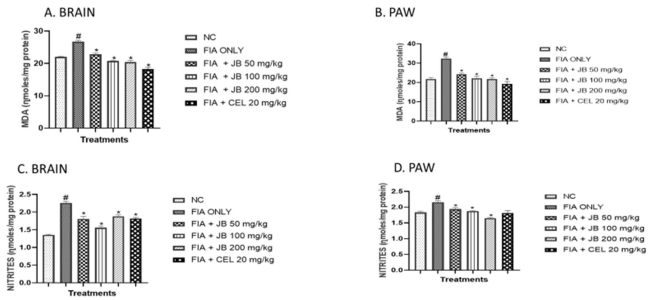

Fig. 4: Effect of JB on oxidative stress markers in the brain and paw tissue (A) MDA level in the brain (B), MDA level in the paw (C), nitrite level in the brain (D), and nitrite level in the paw of FIA mice. Values represent mean ± SEM (n=5). # P < 0.05 vs control, *P < 0.05 vs FIA using one-way ANOVA followed by Tukey's *post hoc* test.

Effect of JB on GSH and CAT Levels in the Brain and Paw Tissues of FIA Mice

There was a significant (p < 0.05) reduction in brain and paw tissue GSH and CAT levels in the FIA-only group as compared to the control. Treatment with JB (50, 100 and 200 mg/kg) and CEL (20 mg/kg) significantly (p < 0.05) increased brain and paw GSH and CAT levels (Fig. 5 A-D) when compared to arthritic control (FIA only).

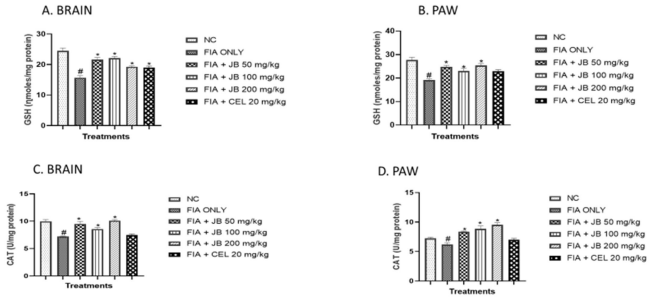

Fig. 5: Effect of JB on oxidative stress markers in the brain and paw tissue (A) GSH level in the brain (B) GSH level in the paw (C) CAT level in the brain (D) CAT level in the paw of FIA mice. Values represent mean ± SEM (n=5). # P < 0.05 vs control, *P < 0.05 vs FIA using one-way ANOVA followed by Tukey's *post hoc* test.

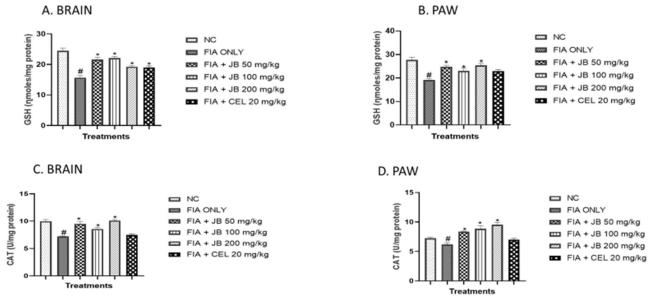

Fig 6: Effect of JB on oxidative stress markers in the brain and paw tissues: (A) SOD level in the brain (B) SOD level in the paw (C) SOD level in the brain (D) SOD level in the paw of FIA mice. Values represent the mean ± SEM (n=5). # P < 0.05 vs control, *P < 0.05 vs FIA using one-way ANOVA followed by Tukey's *post hoc* test.

JB Reduces Brain and Paw Inflammatory Cytokines in FIA Mice

Figures 7A-C showed the effects of JB on inflammatory cytokines in the brain and paw tissues of mice. Mice injected with formaldehyde had a significant increase in TNF-α level in the brain and paw tissues when compared to the control. However, treatment with JB (50, 100 and 200 mg/kg) or CEL (20 mg/kg) significantly ($p<0.05$) decreased the levels of TNF-α in a dose-dependent fashion compared to the arthritic control group (FIA only).Likewise, FIA alone significantly increased the levels of IL-6 when compared with control, while JB treatment and CEL significantly ($p < 0.05$) reduced FIA-induced elevation of IL-6, as shown in Figure 7b.

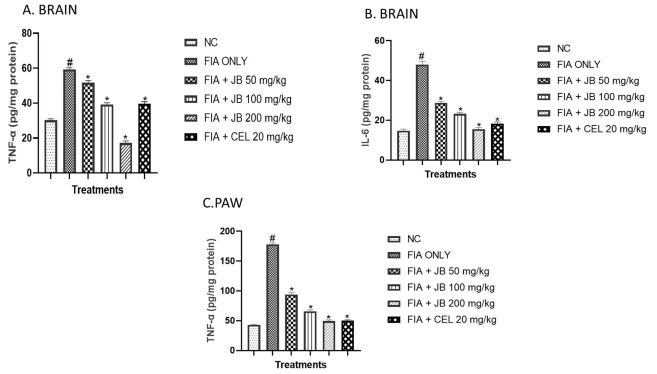

Fig. 7: The effect of JB on inflammatory cytokines in the brain and paw tissues: (A) TNF-α in the brain (B) IL-6 in the brain (C) TNF-α in the paw of FIA mice. Values represent the mean ± SEM (n=5). # $P < 0.05$ vs control, *$P < 0.05$ vs FIA using one-way ANOVA followed by Tukey's *post hoc* test.

Effect of JB on AChE Enzymes in the Brain of FIA Mice

Table 1 showed the effects of JB on neurotransmitter enzymes in the brains of FIA mice. There was significant ($p<0.05$) elevation in AChE in the brain of FIA mice relative to the control group. However, treatment with JB (50, 100 and 200 mg/kg) or CEL (20 mg/kg) significantly ($p<0.05$) reduced AChE when compared with the FIA-alone group.

Table 1: Effect of JB on AChE enzyme in the brain of FIA mice

Treatments	AChE (µmoles/min/mg protein)
NC	1.21 ± 0.02
FIA ONLY	1.31 ± 0.01
FIA + JB 50 mg/kg	1.05 ± 0.04
FIA + JB 100 mg/kg	0.91 ± 0.02
FIA + JB 200 mg/kg	1.09 ± 0.03
FIA + CEL 20 mg/kg	1.20 ± 0.03

Values represent mean ± SEM (n=5). # $P < 0.05$ vs control, *$P < 0.05$ vs FIA using one-way ANOVA followed by Tukey's post hoc test.

DISCUSSION

The findings from this study demonstrated that JB treatment significantly attenuated both neurogenic and inflammatory pain, as evidenced by decreased paw-licking time and frequency, alongside increased thermal hyperalgesia latency. Results also indicate that JB reduced inflammatory responses in mice, as evidenced by a decrease in inflammatory biomarkers TNF-α and IL-6 in FIA.

In this study, our results indicated that JB significantly reduced paw-licking time in the early phase and late phase in a dose-dependent manner when compared to the formaldehyde-induced group. This result implies that JB treatment significantly reduced both neurogenic and inflammatory pain responses induced by formaldehyde injection, suggesting that it possesses both central and peripheral analgesic properties. JB also reduced inflammatory responses induced by subplantar injection of formaldehyde as measured by an increase in arthritic score in the treatment groups when compared with the FIA group, suggesting potential benefits in inflammatory conditions associated with arthritis. Assessing arthritis severity in mouse models, especially via measurement of paw inflammation, is vital for evaluating disease progression and therapeutic intervention (Miyoshi *et al.*, 2018). Scoring involves assessment of paw inflammation based on parameters like redness and swelling, with higher scores indicating severity (Perilli *et al.*, 2015). JB also increased pain sensitivity in the hyperalgesia test in mice with a subplantar injection of formaldehyde when compared to the FIA group and control. Arthritic action of formaldehyde leads to secretion of mediators like prostaglandin, which results in marked permeability and vasodilation. These mediators are responsible for hyperalgesia by stimulating nerve terminals and pain receptors. Hence, hypersensitivity is evoked at the injection site (Manan *et al.,* 2022).

This study demonstrated a reduction in the number of crossed lines in the FIA group compared to the control group. The number of lines crossed was increased in the treatment groups, which suggests that FIA reduced motor behavioural changes which were attenuated by the treatments. The results also indicated that the number of open arm entries in the elevated plus maze was reduced in the FIA group when compared with the control group, which was increased by the JB treatment when compared with FIA alone. Furthermore, the number of closed arm entries was increased by the FIA group, which was mitigated by the JB treatment. Our findings indicated that arthritic mice displayed a motor impairment and anxiety-like symptoms, aligning with previous research (Stubbs *et al.*, 2016; Abbas *et al.,* 2024). A clinical investigation has shown that motor impairments and anxiety are common psychopathologies in individuals with chronic pain, including arthritic pain, and that these issues significantly contribute to a decline in quality of life (Chaurasia *et al.*, 2020).

The early phase of the formaldehyde injection elicited localised inflammation and pain, followed by a phase of tissue-mediated responses (Wheeler-Aceto and Cowan, 1991). Results of this study indicated that the levels of MDA; an indicator of lipid peroxidation) and nitrites, which were elevated by the FIA group, were attenuated by the various treatment groups (the three doses of JB and CEL when compared with the FIA group. The MDA levels were decreased in a dose-dependent manner. Elevated levels of oxidative stress biomarkers, including lipid peroxidation products, have been observed in arthritic patients (Phull *et*

al., 2018; Radu and Bungau, 2021). Therefore, targeting lipid peroxidation may provide therapeutic potential for mitigating joint inflammation and tissue degradation associated with arthritic conditions. Findings that JB reduces FIA-increased levels of MDA and nitrite in the brain and paw tissues of mice suggest antioxidant properties.

Oxidative stress can cause cartilage degradation and inflammation, as well as joint damage. Oxidative stress plays a critical role in the pathology of arthritis, as evidenced by alterations in the antioxidant system and elevated levels of lipid peroxidation in the blood and synovial fluid of affected individuals (Phull *et al.*, 2018; Radu and Bungau, 2021). This result also indicated that JB and CEL improved the level of the antioxidant enzymes, such as GSH, SOD, GST and CAT. The FIA group increased these antioxidant levels, while the control group reduced them. These effects were observed in the brain and paw tissues of the mice. Likewise, a similar attenuating effect was observed in the levels of GST, where the FIA group decreased the levels of GST when compared with the control, and it was elevated by the various groups administered with JB and CEL when compared with the FIA group. The attenuation of FIA-induced depletion of glutathione (GSH), SOD and CAT in brain and paw tissues of mice by JB further suggests it has antioxidant properties. Thus, it might be suggested that the antioxidant activity of JB plays vital roles in the joint protection in FIA-treated mice.

This study indicated that JB decreased levels of pro-inflammatory cytokines, IL-6 and TNF-α in FIA mice's brain and paw tissues. The results of this study showed a significant increase in the levels of TNF-α in the brain and paw tissues of mice in the FIA group when compared with the control, and this was attenuated by the various groups treated with JB and CEL, which showed decreased TNF-α levels when compared with the FIA group. Similarly, the levels of IL-6 was increased by the FIA group when compared with the control, and this was mitigated by treatment with JB, as evidenced by decreased levels of IL-6 when compared with the FIA group. This inhibitory effect on the release of TNF-α and IL-6 in FIA is consistent with results obtained in an immunological model of CFA-induced arthritis (Omorogbe *et al.*, 2018; Abbas *et al.*, 2024).

The activities of AChE in the brain tissues of FIA mice were investigated. Elevated AChE activity implies a reduced amount of acetylcholine, a neurotransmitter crucial in motor disorders, learning, and memory (Ali and Zainal, 2025). This study demonstrated that FIA increased AChE activity in mice's brains, suggesting a decrease in cholinergic function, which JB mitigated. The level of AChE activity was increased in the group exposed to FIA alone without treatment when compared with the control group that wasn't exposed to FIA at all. This elevation was seen to be reduced by the various groups administered with JB and CEL after exposure to FIA, which indicates that the treatment groups reduced the levels of AChE activity when compared with the FIA alone group. Research indicates that AChE inhibitors can suppress inflammation in arthritis models and the relationship between AChE levels and arthritis (Van Maanen *et al.,* 2009). These studies highlight a significant association between AChE levels and arthritis, suggesting that modulation of AChE activity could be a potential therapeutic strategy for managing arthritis (Ali *et al.*, 2021). Previous studies have shown the presence of potent antioxidant, anti-inflammatory, and neuroprotective compounds in JB, including luteolin, naringenin, apigenin, and 3-deoxy-anthocyanidins (Omorogbe *et al.*, 2018; Adebesin *et al.*, 2024).

Conclusion

The findings of this study reveal that formaldehyde administration led to increased paw licking time and higher arthritic scores, as well as decreasing hyperalgesia latency. It also elevated the number of line crossings in the open field test and decreased closed arm entries while increasing open arm entries in the behavioural assessment. Additionally, formaldehyde also reduced antioxidant levels (SOD, CAT, GSH, and GST) and increased MDA and nitrite concentrations, as well as inflammatory markers such as TNF-α and IL-6. JB reduced arthritis scores and anxiety-like behaviours in both open-field and elevated plus maze tests, as well as thermal hyperalgesia and neuroinflammation in FIA in mice, supporting its widely recognised benefit in people with arthritic illness. These findings suggest that JB possesses both central and peripheral analgesic properties due to the mitigatory effect observed in the paw licking times and frequency, as well as in the open field test where locomotion was improved against damages caused by FIA. Additionally, JB treatment alleviated inflammation by mitigating the increased levels of inflammatory biomarkers caused by FIA exposure. These results indicate that JB may offer therapeutic benefits for managing painful inflammatory conditions. The potential of JB to improve motor impairments and anxiety in FIA-induced arthritis in mice may indicate therapeutic effects in enhancing the quality of life of people suffering from chronic arthritic pain.

DECLARATION

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None declared.

Conflict of Interest

None declared.

Ethical Approval

All animal experiments were conducted in accordance with the relevant guidelines and regulations, including the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals. This study is part of the ongoing investigations of the effects of Jobelyn on various inflammatory models in rodents with the experimental procedures approved by the University of Ibadan Animal Care and

Use Research Ethics Committee (approval number UI-ACUREC/084-0923/8).

Consent to Participate and Publish Data
Not Applicable.

Authors Contributions
ANA: Conceptualisation, data acquisition, data analysis, methodology, supervision, and writing (review and editing). GAA: Data analysis, writing (original draft), and writing (review and editing). AMO, OOM, DJA, JMO, HAA, OA, IRA, AMI: Data acquisition; Data analysis; Methodology. AMA: Conceptualisation; Data acquisition; Data analysis; Methodology; Writing (review and editing).

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