



Jobelyn[®] attenuates inflammatory responses and neurobehavioural deficits associated with complete Freund-adjuvant-induced arthritis in mice



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ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects the physical and psychosocial wellbeing of the patients and a major cause of work disability. Current drugs for its treatment only provide palliative effect, as cure for the disease still remains elusive. Jobelyn[®] (JB), a potent anti-oxidant and anti-inflammatory dietary supplement obtained from *Sorghum bicolor*, has been claimed to relieve arthritic pain. Thus, this study was designed to evaluate its effect on inflammatory and biochemical changes as well as neurobehavioural deficits associated with complete Freund-adjuvant (CFA)-induced arthritis in mice. The effect of JB (50, 100 and 200 mg/kg) on inflammatory oedema, neurobehavioural deficits, levels of biomarkers of oxidative stress and inflammatory cytokines (tumor necrosis factor-alpha and interleukin-6) induced by 0.1 mL of CFA (10 mg/mL) was evaluated in male Swiss mice. Oral administration of JB (100 and 200 mg/kg) reduced inflammatory paw volume and reversed sensorimotor deficits induced by CFA. JB also reduced pain episodes, anxiety and depressive-like symptoms in CFA-mice. The increased level of oxidative stress in the joint and brain tissues of CFA-mice was reduced by JB. It also decreased tumor necrosis factor-alpha and interleukin-6 levels induced by CFA in the joint tissue of mice. These findings suggest that Jobelyn[®] attenuates inflammatory responses induced by CFA in mice via inhibition of oxidative stress and release of inflammatory cytokines. The ability of JB to attenuate CFA-induced nociception, sensorimotor deficits and depressive-like symptom suggests it might improve the quality of life of patients with arthritic conditions.

1. Introduction

Rheumatoid arthritis (RA) is a common chronic inflammatory disease that affects the physical, psychological and social wellbeing of the patients and a major cause of work disability in people over 50 years of age across the globe [1,2]. RA is characterized by chronic inflammation of the synovial membrane, pain and restricted joint movement [3–5]. This results in disability and high degree of morbidity that often leads to disturbed daily life of the patients and also known to shorten the lifespan of the patients [1,5]. However, the symptoms of RA are not always confined to the joints, as it can cause extra-articular manifestations such as cardiovascular and neurological complications [6–8]. The incidence of RA increases with age, with women being affected 25-times higher than men [9,10]. The risk of the disease appears to be greatest for women between 40 and 50 years of age, and for men somewhat later, which makes it to be an age and sex-related disorder [1]. The chronic nature of RA and the understanding that the disease is

incurable may cause a wide range of emotional reactions such as grief, worry, fatigue, dysthymia, anger and social withdrawal, which are the major components of depression [11,12]. Anxiety and depression have been reported as common features seen in patients with RA, with higher prevalence than that of the normal population [13]. Patients with symptoms of depression often exhibit feelings of sadness, helplessness and loss of pleasure or interest and sometimes suicidal tendency [8].

Although the etiology of RA still remains largely unknown, infiltrations of inflammatory cells into the joint play a prominent role in the mediation of tissue destruction that characterized the pathology of the disease [9,10]. In fact, the increase in the activity of inflammatory cells (leukocytes) plays a prominent role in the pathogenesis of the disease [14,15]. The initiation and progression of the disease are majorly related to the migration of inflammatory cells to the inflamed joint in response to the release of chemical mediators such as cytokines, prostaglandins, and leukotrienes [15,16]. The activity of the leukocytes results in the release of free radicals and other cytotoxic substances

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including proinflammatory cytokines, which initiate and propagate bone destruction seen in patients with RA [15–17]. The extent of macrophage infiltration into the synovial, for example has been shown to correlate with the severity of the disease and its progression [2,18].

Current approach to the management of RA focuses on the alleviation of symptoms as cure for the disease still remains elusive. The major goal of treatment is to reduce pain, decrease inflammation, and improve on the quality of life of the patients [19]. The non-steroidal anti-inflammatory drugs and steroids are used to relieve inflammatory pains but their prolonged administration is beset with several adverse effects [15]. Moreover, the disease-modifying anti-rheumatic drugs also have limited clinical efficacy and serious adverse effects [1,19]. Thus, there is an urgent need to continue to search for alternative agents for the treatment of the disease. However, a number of medicinal plants with multiple sites of action that could target both the inflammatory and oxidative pathways that are the key elements involved in the pathology of RA are being investigated as new medicines for the disease [16–21].

Jobelyn[®] (JB) is an African-based food supplement derived from the polyphenol-rich leaf sheath of *Sorghum bicolor* (L) Moench (family: Poaceae), a plant reputed for its nutritional and medicinal qualities across the globe [23]. *Sorghum bicolor*, commonly known as millet, has been used for over hundred years for the treatment of a variety of ailments in African traditional medicine [23]. Jobelyn[®] is manufactured by Health Forever Products Ltd, Lagos, Nigeria and available as capsules (250 mg per capsule) in most pharmaceutical outlets in Nigeria and also abroad including United States of America. JB has gained local and international recognition for the treatment of moderate to severe anaemia (as in sickle cell patients), cancer and HIV/AIDS [24,25]. It is also widely used across the globe to combat stress and to restore the much needed energy during the periods of recovery from debilitating diseases [24,25]. The manufacturer also claimed that JB is helpful in arthritis and neurological disorders such as psychosis, stroke and convulsions. In addition, JB is known to boost the immune system, thereby enhancing the body's defensive mechanisms in response to stress, infections or debilitating illnesses like RA [24,25]. The recommended dose by the manufacturer is 1 or 2 capsules (1–3 times daily) depending on the severity of the ailments. The major active ingredients in JB include proanthocyanidins, anthocyanidins, apigenidins, proapigenidins, apigenins, luteolins and naringenins [23,26]. Most of these biologically active compounds have been found to exhibit a wide range of pharmacological activities including neuroprotection, anti-nociception, anti-inflammatory and anti-amnesic activities [27–30]. Previous studies have confirmed the anti-anemic effect of JB, as it was shown to boost haemoglobin concentrations and packed cell volume in laboratory animals [24,25]. In our previous studies, we have reported that JB demonstrated anti-inflammatory effect in carrageenan models and stabilized erythrocyte membrane [31]. Moreover, Benson et al. [26] also reported that JB demonstrated anti-inflammatory activity in culture cells via inhibition of infiltrations of WBC, release of inflammatory cytokines and free radical formation. However, no investigations have been carried out to evaluate the effects of JB on complete Freund-adjuvant-induced arthritis that closely mimics the pathology of RA. This study was therefore designed to investigate the effects of Jobelyn[®] on the inflammatory and biochemical changes as well as neurobehavioural deficits associated with complete Freund-adjuvant-induced arthritis in mice.

2. Materials and methods

2.1. Experimental animals

Male Swiss mice (22–26 g) used in this study were obtained from the Central Animal House, University of Ibadan. The animals were kept in plastic cages at room temperature with 12:12 h light-dark cycle and were allowed free access to commercial rodent pellet diet and water *ad*

libitum. They were acclimatized for one week before commencement of experiments and all the experimental procedures in this study were performed in compliance with the NIH Ethical Guidelines for the Care and Use of Laboratory Animals.

2.2. Drugs and chemicals

Jobelyn[®]-JB (Health Forever Products Ltd, Lagos, Nigeria), Celecoxib-CEL (Medico Remedies PVT), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB; Sigma Aldrich, USA), trichloroacetic acid (Sigma Aldrich, USA), thiobarbituric acid (Sigma Aldrich, USA), complete Freund-adjuvant-CFA (Santa Cruz Biotech., USA), sodium carbonate (BDH Poole, England), potassium carbonate (BDH Poole, England), and sodium chloride (BDH Poole, England) were used in this study.

2.3. Drug preparation and treatment

Jobelyn[®] and celecoxib were dissolved in saline before use and were administered orally. The doses of JB used in the study were chosen based on the results obtained from previous investigations [31]. The dose of CEL (20 mg/kg) was selected according to existing literature [32].

2.4. Effect of Jobelyn[®] on inflammatory responses in complete Freund-adjuvant-induced arthritis model

The effect of JB on inflammatory responses in complete Freund-adjuvant-induced arthritis was carried out according to the method previously described [33]. Briefly, mice were allotted into 6 groups (n = 6) and baselines left hind paw volume were measured on day 0. Mice in group 1, which served as vehicle-control were given saline (10 mL/kg, p.o.), group 2 serving as CFA-control also received saline (10 mL/kg, p.o.), group 3 had CEL (20 mg/kg, p.o) while groups 4–6 received JB (50, 100 and 200 mg/kg, p.o.) daily for 14 days. However, treatments were given to mice in groups 2–6 on day 1 after intraplantar injection of 0.1 mL of CFA containing 10 mg/mL of heat killed *Mycobacterium tuberculosis* into the left hind paw of the animals. The increases in paw volume were measured using a plethymometer on days 6, 9 and 14. The changes in paw volume were calculated as the differences between the paw volume at day 0 and 6, 9 and 14 after injection of CFA.

2.5. Neurobehavioural tests

The neurological deficits such as postural instability, anxiety, depression and altered motor activity that often characterized the pathology of RA were evaluated in this study. The neurobehavioural tests: test for spontaneous motor activity (SMA), postural stability, anxiety and depressive-like behaviours were carried out in this sequence on day 14 post-injection of CFA.

2.5.1. Test for spontaneous motor activity

The test for SMA was done on day 14 post-injection of CFA using activity cage (Ugo Basile, Italy). Mice in each group were placed individually in the activity cage and the SMA was recorded as activity counts per 5 min.

2.5.2. Evaluation of postural stability

The sensorimotor functions of the animals were assessed immediately after testing for SMA using the walk beam test (WBT). The WBT is used to evaluate fine motor coordination and balance of naïve animals or those under the influence of drugs. The beam apparatus composed of a piece of wood of length 100 cm, width 2 cm and elevated to a height of 40 cm. The beam is coated with black paint and marked at 5 cm and 1 cm intervals. The WBT consists of two phases; the trial and test periods. In the test phase, the mouse was placed at one end of the

beam and allowed to move freely on the beam after securing its grip on it for a period of 2 min. The beam was cleaned with 70% ethanol and allowed to dry between each trial. The test phase was done after the trial session and the total distance travelled, number of foot slips and turns were recorded for a period of 2 min. The total distance travelled (cm) referred to the number of times the animal goes over the beam within the 2 min test period multiplied by its current position on the beam. Foot slip occurs whenever the back foot of the animal slips off the beam. Meanwhile, turning behaviour occurs when the animal on reaching the end of the beam turns to the opposite direction.

2.5.3. Test for anxiety-like behaviour using light/dark box

The light-dark box was used in this study to evaluate the effect of CFA-induced anxiety-like behaviour according to the procedure previously described [34]. Briefly, immediately after the WBT, the animals were placed individually at the center of the bright compartment and allowed to explore the apparatus for a period of 5 min [34]. The total time spent (s) in the light and dark compartments were recorded for a period of 5 min. The maze was cleaned with a solution of 70% ethyl alcohol and permitted to dry between tests to prevent odor bias.

2.5.4. Test for depression using tail suspension test (TST)

The TST is widely used as a screening procedure for evaluation of novel compounds for anti-depressant property in rodents [35]. Mice were suspended 50 cm above the floor, with the help of an adhesive tape placed approximately 1 cm from the tip of the tail immediately after the light/dark box test. The period of immobility (s) was then measured for 4 min after the 2 min delay and the animals were considered immobile whenever they remained motionless and hung passively [35].

2.5.5. Test for mechanical hyperalgesia

The nociceptive response to application of a clip on the inflamed hind paw of CFA-mice as model of mechanical hyperalgesia was evaluated on day 15 post-CFA injection. Briefly, inflamed hind paws of the animals were individually clipped with a clip and the time (s) it took the animal to bite the clip, an index of nociception was recorded. However, a cutoff time of 60 s was imposed to prevent tissue damage and the clip was immediately removed whenever the animals show sign of pain. The animals in the vehicle-control group that were not pre-treated with CFA were also subjected to the pain episode in a similar manner.

2.6. Preparations of tissues for biochemical studies

The animals were sacrificed through ether anaesthesia immediately after testing for nociception and the joint, and brain tissues were isolated, weighed and kept in 10%w/v phosphate buffer (0.1 M, pH 7.4). The joint tissues or the whole brains of the mice were homogenized with the 10% w/v phosphate buffer and the supernatant stored at -20°C until use for the different biochemical assays and enzyme immunoassay.

2.6.1. Determination of glutathione (GSH) concentration

The concentration of GSH in the joint tissue and brain supernatant of mice in each group was determined using the method of Moron et al. [36]. Briefly, equal volume (0.4 mL) of the tissue homogenate and 20% trichloroacetic acid (TCA) (0.4 mL) were mixed and then centrifuged using a cold centrifuge at 10,000 rpm at 4°C for 20 min. The supernatant (0.25 mL) was added to 2 mL of 0.6 mM DTNB reagent and the final volume was made up to 3 mL with phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm against blank reagent using spectrophotometer. The concentrations of GSH in the joint tissues or brain tissues were expressed as micromoles per gram tissue ($\mu\text{mol/g}$ tissue).

2.6.2. Estimation of concentration of malondialdehyde (MDA)

The MDA levels in the joint and brain tissues of mice in each group were determined as previously described [37]. Briefly, 0.5 mL of distilled water and 1.0 mL 10% TCA were added to 0.5 mL of the joint tissue and brain supernatant and centrifuged at 3000 rpm for 10 min. Then, 0.1 mL thiobarbituric acid (TBA) (0.375%) was added to the supernatant. The mixture was then placed in a water bath at 80°C for 40 min and then cooled to room temperature. Upon cooling, the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The MDA concentration was calculated using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and values were expressed as $\mu\text{moles/g}$ tissue.

2.6.3. Estimation of superoxide dismutase (SOD) activity

The levels of SOD activity were determined as previously described [38]. Briefly, 1 mL of the joint and brain tissues supernatant were diluted with 9 mL of distilled water to make a 1 in 10 dilution. An aliquot of 0.2 mL of the diluted sample was added to 2.5 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction was started by addition of 0.3 mL of freshly prepared 0.3 mM adrenaline to the mixture, which was quickly mixed by inversion. The reference cuvette contains 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 every 30 s for 150 s.

2.6.4. Estimation of the concentrations of tumor necrosis factor-alpha and interleukin-6 in the joint tissues

The concentrations of tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) in the joint tissue supernatant were estimated using ELISA MAXTM Deluxe kit (BioLegend, USA) according to the manufacturer's instructions.

2.7. Statistical analysis

The data were analyzed using Graph Pad Prism software version 5.0 and expressed as mean \pm standard error of mean (S.E.M). Statistical analysis was done using one-way or two-way ANOVA, followed by Newman-Keuls post-hoc test or Bonferroni post hoc test for multiple comparisons as appropriate. P values less than .05 were considered statistically significant.

3. Results

3.1. Jobelyn[®] reduces inflammatory responses induced by complete Freund-adjuvant

The inflammatory responses induced by intraplantar injection of CFA as measured by increase in paw volume are presented in Fig. 1. Two-way ANOVA revealed that intraplantar injection of CFA produced significant differences in paw volume on day 6 [F (6, 36) = 13.75;

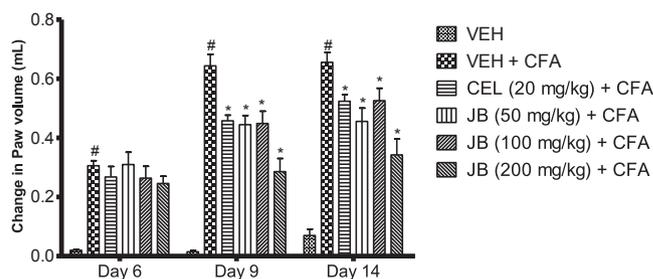


Fig. 1. Effects of Jobelyn[®] (JB) and celecoxib (CEL) on complete Freund-adjuvant (CFA)-induced increase in paw volume in mice. Vertical bars represent mean \pm S.E.M for 6 animals per group. #p < .05 compared to vehicle (VEH)-treated group and *p < .05 compared to VEH + CFA-treated group (Two-way ANOVA followed by Bonferroni post hoc test for multiple comparison).

Table 1
Effect of Jobelyn[®] on spontaneous motor activity of mice treated with complete Freund-adjuvant.

Treatment	Activity Counts/5 min
Vehicle	233.3 ± 30.88
Vehicle + CFA	319.3 ± 26.47
CEL (20 mg/kg) + CFA	323.4 ± 46.59
JB (50 mg/kg) + CFA	375.7 ± 47.72
JB (100 mg/kg) + CFA	286.6 ± 18.78
JB (200 mg/kg) + CFA	284.0 ± 56.0

Values represent the mean ± S.E.M for 6 animals per group; $p > .05$.

$p < .0001$], day 9 [F (6, 36) = 42.60; $p < .0001$] and day 14 [F (6, 36) = 30.55; $p < .0001$]. However, the increased paw volume induced by CFA was significantly ($p < .05$) reduced by JB (100 and 200 mg/kg, p.o.) or CEL (20 mg/kg) (Fig. 1).

3.2. Effect of Jobelyn[®] on spontaneous motor activity in complete Freund-adjuvant-treated mice

Table 1 showed the effect of JB on SMA as assessed by the activity cage (Ugo Basile, Italy) in CFA-mice. One-way ANOVA showed that there were no significant differences between treatment group: activity counts [F(6, 36) = 1.471; $p = .2433$]. As shown in Table 1, CFA increased activity counts but was not significant ($p > .05$) when compared with control. Moreover, neither JB nor CEL significantly alters the activity counts in comparison with CFA group (Table 1).

3.3. Jobelyn[®] ameliorates sensorimotor deficits induced by complete Freund-adjuvant

The effect of JB on sensorimotor deficits induced by CFA as measured by the walk beam paradigm is depicted in Table 2. One-way ANOVA showed that there were significant differences between treatment group: distance travelled [F(6, 36) = 2.472; $p = .00768$], number of foot slips [F(6, 36) = 4.892; $p = .0066$] and number of turns [F(6, 36) = 4.159, $p = .0130$]. Post-hoc analysis by Newman–Keuls test revealed that CFA produced a significant ($p < .05$) decreases in distance travelled by mice in the horizontal beam relative to control. As shown in Table 2, CFA significantly ($p < .05$) increased the number of foot slips from the horizontal beam and also impaired the number of turns

Table 2
Effect of Jobelyn[®] (JB) and celecoxib (CEL) on sensorimotor deficits induced by complete Freund-adjuvant (CFA) in mice.

Treatment	Distance travelled (cm)	Number of foot slips	Number of turns
Vehicle	186.3 ± 21.45	0.0 ± 0.0	4.500 ± 1.190
Vehicle + CFA	137.5 ± 46.26 [#]	3.000 ± 0.41 [#]	1.500 ± 0.65 [#]
CEL + CFA	225.5 ± 29.49 [*]	0.2500 ± 0.25 [*]	4.000 ± 0.41 [*]
JB (50 mg/kg) + CFA	131.3 ± 39.86	2.000 ± 0.82	1.250 ± 0.25
JB (100 mg/kg) + CFA	192.5 ± 30.86 [*]	1.500 ± 0.65 [*]	2.500 ± 0.29 [*]
JB (200 mg/kg) + CFA	322.5 ± 77.50 [*]	1.500 ± 0.50 [*]	2.500 ± 0.50 [*]

Each value represents the mean ± S.E.M for 6 animals per group.

[#] $p < .05$ compared to vehicle.

^{*} $p < .05$ compared to vehicle + CFA-treated group (One-way ANOVA followed by Newman Keuls test). The sensorimotor functions were performed by placing the animal on horizontal beam of length 100 cm and the total distance travelled, number of foot slips and turns were recorded for a period of 2 min. The total distance travel referred to the number of times the animal goes over the beam within the 2 min test period multiplied by its current position on the beam. Foot slip occurs whenever the back foot of the animal slips off the beam while turning behaviour occurs when the animal on reaching the end of the beam turns to the opposite direction.

Table 3
Jobelyn[®] (JB) and celecoxib (CEL) reduced anxiety-like behaviour induced by complete Freund-adjuvant (CFA) in mice.

Treatment	Duration (s) in the light compartment	Duration (s) in the dark compartment
Vehicle	122.36 ± 20.76	106.10 ± 7.52
Vehicle + CFA	85.31 ± 6.12 [#]	205.20 ± 24.61 [#]
CEL + CFA	243.68 ± 11.31 [*]	54.80 ± 10.41 [*]
JB (50 mg/kg) + CFA	232.3 ± 4.06 [*]	62.57 ± 2.6 [*]
JB (100 mg/kg) + CFA	226.2 ± 4.84 [*]	71.40 ± 4.62 [*]
JB (200 mg/kg) + CFA	207.23 ± 3.30 [*]	87.13 ± 3.10 [*]

Values represent the mean ± S.E.M for 6 animals per group.

[#] $p < .05$ compared to vehicle-treated group.

^{*} $p < .05$ compared to vehicle + CFA group (One-way ANOVA followed by Newman Keuls test).

made by mice when compared with control. However, JB (100 and 200 mg/kg) or CEL (20 mg/kg) attenuated the sensorimotor deficits produced by CFA as shown by increased distance travelled, reduced incidence of foot slips and increased turning behaviour in the WBTT (Table 2).

3.4. Jobelyn[®] reduces anxiety-like behaviour induced by complete Freund-adjuvant

The effect of JB on anxiety-like behaviour induced by CFA in mice is presented in Table 3. One-way ANOVA revealed that there were significant differences between treatment groups: time spent in light compartment [(F6, 36) = 10.201; $p < .0001$] and time spent in dark compartment [(F6, 36) = 8.605; $p < .0001$] when compared with control. Post-hoc analysis by Newman–Keuls test showed that JB (50, 100 and 200 mg/kg, p.o.) or CEL (20 mg/kg) significantly ($p < .05$) attenuated the anxiety-like behaviour induced by CFA in mice as evidenced by increased time spent in the light compartment of the box (Table 3).

3.5. Jobelyn[®] attenuated depressive-like symptom induced by complete Freund-adjuvant

The effect of JB on depressive-like behaviour induced by CFA in the TST in mice is presented in Fig. 2. CFA-mice exhibited significant [F (6, 36) = 13.63; $p < .0001$] increase in the period of immobility when compared with control. However, JB (50, 100 and 200 mg/kg) produced a significant and dose-related decreases in the immobility time relative to CFA group (Fig. 2).

3.6. Effect of Jobelyn[®] on complete Freund adjuvant-induced mechanical hyperalgesia

One-way ANOVA showed that there were significant differences between treatment group: latency to nociceptive behaviour [F(6, 36) = 4.956; $p = .0015$]. Post-hoc analysis by Newman–Keuls test revealed that intraplantar injection of CFA produced significant ($p < .05$) decreases in pain threshold in the inflamed paw of CFA-mice relative to control (Fig. 3). However, CEL (20 mg/kg, p.o) but not JB (50–200 mg/kg, p.o) reduced the reaction time to pain stimulus in a significant manner (Fig. 3).

3.7. Jobelyn[®] decreases MDA concentrations in the joint and brain of CFA-mice

The effects of JB on the concentrations of MDA in the joint and brain tissue homogenates of CFA-mice is shown in Fig. 4. One-way ANOVA

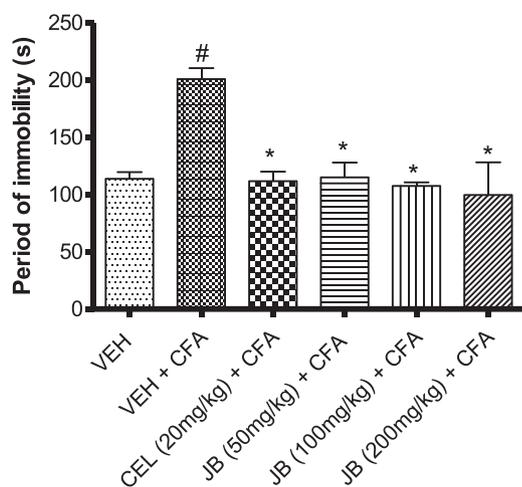


Fig. 2. Effect of Jobelyn® (JB) and celecoxib (CEL) on immobility time in complete Freund- adjuvant (CFA)-treated mice in the tail suspension test. The vertical bars represent the mean ± S.E.M for 6 animals per group. #p < .05 compared to vehicle (VEH)-treated group and *p < .05 compared to VEH + CFA-treated group (One-way ANOVA followed by Newman Keuls test).

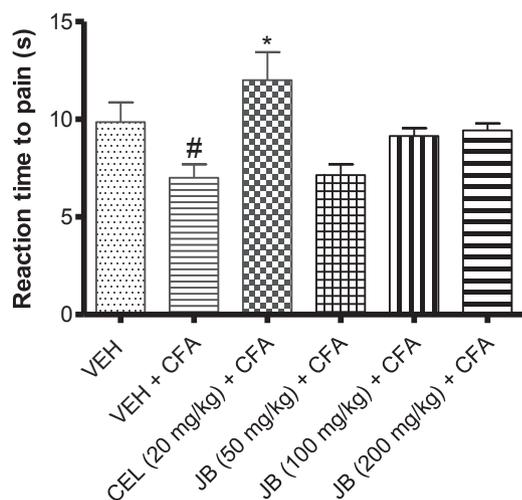


Fig. 3. Effect of Jobelyn® (JB) and celecoxib (CEL) on complete Freund-adjuvant (CFA)-induced mechanical hyperalgesia in mice. #p < .05 compared to vehicle (VEH) and *p < .05 compared to VEH + CFA-treated group (One-way ANOVA followed by Newman Keuls test).

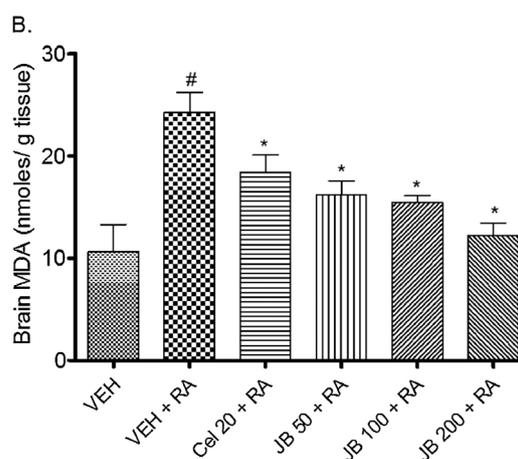
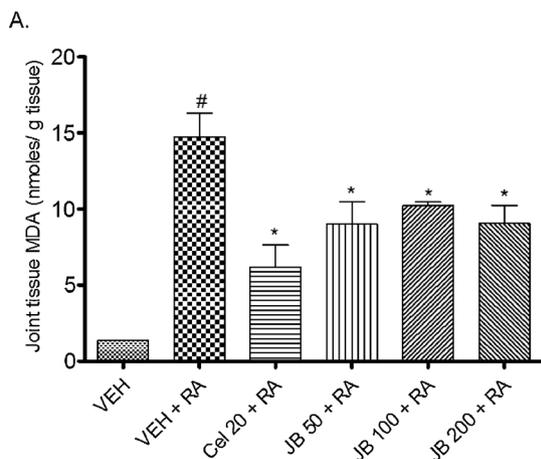


Fig. 4. Effect of Jobelyn® (JB) and Celecoxib (CEL) on MDA levels in the joint tissue (plate A) and brain tissue (B) of mice injected with complete Freund-adjuvant (CFA). Each value represents the mean ± S.E.M for 6 animals per group. #p < .05 compared to vehicle (VEH)-treated group and *p < .05 compared to VEH + CFA-treated group (One-way ANOVA followed by Newman Keuls test).

showed that there were significant differences between treatment groups: MDA joint tissue level [F (6, 36) = 14.81; p < .0001] and Brain MDA level [F (6, 36) = 7.906; p < .0004]. Post-hoc analysis by Newman-Keuls test revealed that CFA produced significant (p < .05) elevation of the concentrations of MDA in the joint and brain tissues relative to controls. However, oral administration of JB (50, 100 and 200 mg/kg) or CEL (20 mg/kg) significantly reduced the level of MDA in the joint and brain tissues in comparison with CFA group (Fig. 4).

3.8. Jobelyn® attenuates complete Freund adjuvant-induced GSH depletion in the joint and brain tissues of mice

The effects of JB on the concentrations of GSH in the joint and brain tissues of CFA-treated mice are shown in Fig. 5. As shown in Fig. 5, oral administration of JB (50, 100 and 200 mg/kg) or CEL (20 mg/kg) attenuated CFA-induced depletion of the concentrations of GSH in the joint tissue [F(6, 36) = 13.48; p < .0001] and brain tissue [F(6, 36) = 6.059; p < .0019].

3.9. Effect of Jobelyn® on the activity of SOD in the joint and brain tissues of CFA-mice

The effect of JB on the activity of SOD in the joint and brain tissues of CFA-mice is presented in Fig. 6. One-way ANOVA showed that there were significant differences between treatment groups: joint tissue [F (6,36) = 15.90; p < .0001] and brain tissue [F(6, 36) = 9.662; p = .0007] SOD activity. However, the reduced SOD activity induced by CFA in these tissues was elevated by JB (100 and 200 mg/kg) or CEL (20 mg/kg) in a significant (p < .05) manner (Fig. 6).

3.10. Jobelyn® decreases the level of inflammatory cytokines (interleukin 6 and tumor necrosis factor-α) in the joint tissue of CFA-mice

The effect of JB on the concentrations of inflammatory cytokines (TNF-α and IL-6) induced by CFA in the joint tissue of mice is presented in Figs. 7 and 8. One-way ANOVA showed that there were significant differences between treatment groups: IL-6 level [F (6, 36) = 11.87; p = .0002] and TNF-α level [F (6, 36) = 86.49; p < .0001]. However, the increased levels of IL-6 and TNF-α in the joint tissue of mice was significantly (p < .05) attenuated by JB (50, 100 and 200 mg/kg) or CEL (20 mg/kg) when compared with CFA group (Figs. 7 and 8).

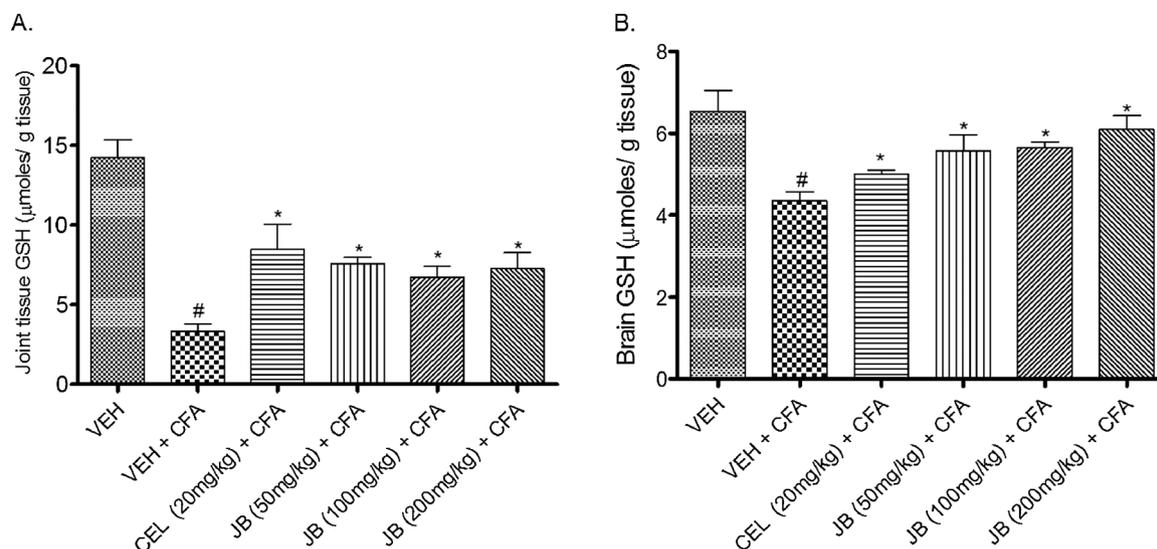


Fig. 5. Effect of Jobelyn[®] on glutathione concentrations in the joint tissue (A) and brain tissue (B) of mice injected with complete Freund-adjuvant (CFA). Each vertical bar represents the mean ± S.E.M for 6 animals per group. [#]p < .05 compared to vehicle (VEH)- treated group and ^{*}p < .05 compared to VEH + CFA-treated group (One-way ANOVA followed by Newman Keuls test).

4. Discussion

The results of this study showed that JB reduces inflammatory and nociceptive responses induced by intraplantar injection of CFA in mice. In the walk beam test, JB ameliorated the sensorimotor deficits induced by CFA. Moreover, the anxiety- and depressive-like behaviours induced by CFA were reduced by JB. In the biochemical studies, JB or CEL attenuated CFA-induced increases in oxidative stress in the joint and brain tissues of mice. In addition, CFA-induced increase in the levels of IL-6 and TNF-α in the joint tissue of mice was also reduced by JB or CEL.

CFA-induced arthritis in rodents has been shown to replicate most of the clinical and pathological features of RA and thus is the most commonly used model for the detection of novel compounds with anti-arthritic activity [40–42]. Previous studies have shown that unilateral intraplantar injection of CFA triggers inflammatory responses in rodents [41]. Chemical mediators like IL-6, IL-1 and TNF-α and prostaglandins have been implicated in the inflammatory effect of CFA [39,40,42]. IL-6, IL-1 and TNF-α, for examples, are known to play

prominent roles in joint inflammation because they mediate the formation of other inflammatory mediators such as prostaglandins, nitric oxide, matrix metalloproteinases and C-reactive protein [2,43]. Specifically, IL-6 has been implicated in the upregulation of adhesion molecules, activation of osteoclasts and activation of B cells [2,43–45]. The increased expression of adhesion molecules on endothelial cells wall, of course, produce recruitment of more inflammatory cells to the joint whose activity leads to further release of cytotoxic metabolites such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Thus, the findings that JB reduced inflammatory oedema induced by CFA suggest that it might offer some beneficial effects in arthritic conditions. This suggestion is further supported by the findings from the biochemical studies, which showed that JB significantly reduced the levels of IL-6 and TNF-α, the prime cytokines known for the induction and propagation of joint inflammation [2,39–43]. The inhibition of the release of these pro-inflammatory cytokines suggest that JB might also affect the levels of other mediators especially prostaglandins at the site of inflammation. It is worthy to note that prostaglandins are known for their ability to potentiate other inflammatory mediators and thus

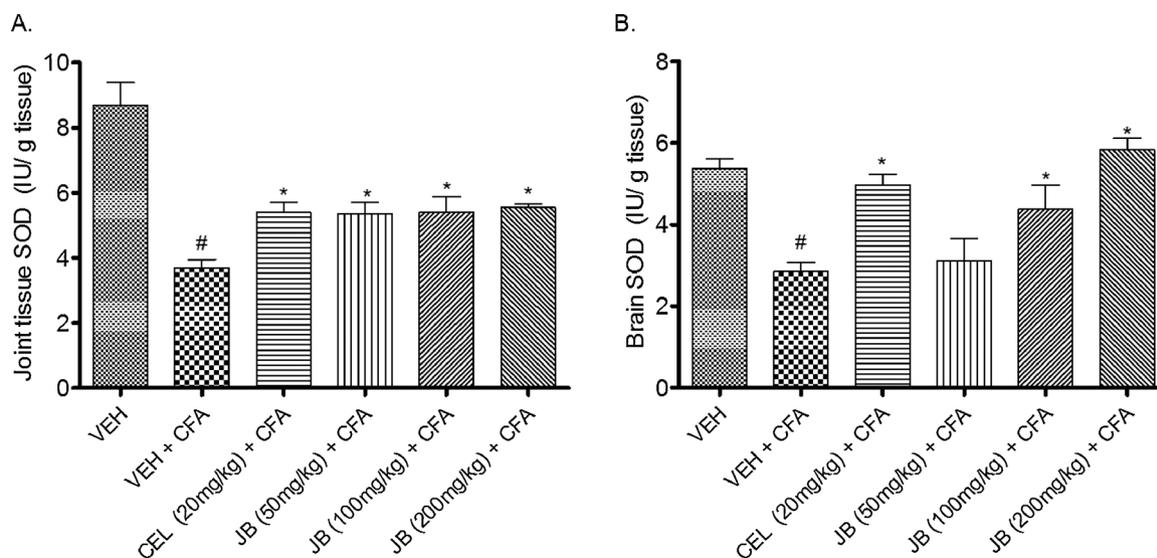


Fig. 6. Effect of Jobelyn[®] on superoxide dismutase activity in the joint tissue (A) and brain tissue (B) of mice injected with complete Freund-adjuvant (CFA). Vertical bars represent the mean ± S.E.M for 6 animals per group. [#]p < .05 compared to vehicle (VEH) and ^{*}p < .05 compared to VEH + CFA group (One-way ANOVA followed by Newman Keuls test).

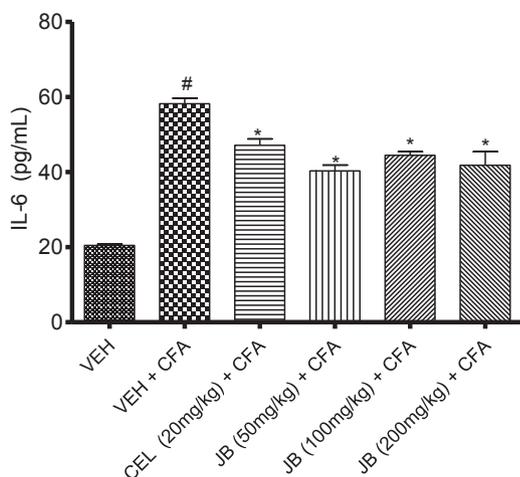


Fig. 7. Effect of Jobelyn® (JB) and celecoxib (CEL) on the levels of interleukin 6 (IL-6) in the joint tissue of mice treated with complete Freund-adjuvant (CFA). Vertical bars represent the mean \pm S.E.M for 6 animals per group. # $p < .05$ compared to vehicle (VEH)-treated group and * $p < .05$ compared to VEH + CFA-treated group (One-way ANOVA followed by Newman Keuls test).

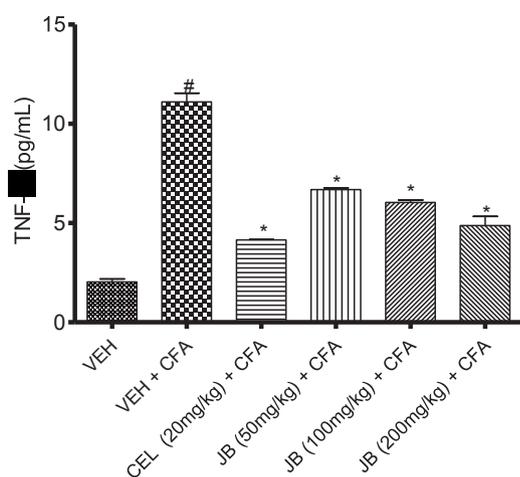


Fig. 8. Effect of Jobelyn® (JB) and celecoxib (CEL) on the levels of tumor necrosis factor alpha (TNF- α) in the joint tissue of mice treated with complete Freund-adjuvant (CFA). Vertical bars represent the mean \pm S.E.M for 6 animals per group. # $p < .05$ compared to vehicle (VEH)-treated group and * $p < .05$ compared to VEH + CFA-treated group (One-way ANOVA followed by Newman Keuls test).

contribute significantly to the progression of chronic inflammatory diseases [2]. Previous studies have also confirmed that JB demonstrated anti-inflammatory activity and inhibited the release of inflammatory mediators from culture cells [26]. Moreover, luteolin, a major constituent of JB was shown to inhibit nuclear factor- κ B (NF- κ B), a major transcription factor in immune cells, which further support the potential benefit of JB in chronic inflammatory diseases [46]. However, more studies are needed to establish the relevance of these findings in the anti-arthritis effect of JB demonstrated in this study.

Increased oxidative stress and nitric alteration have also been reported in the inflammatory responses produced by CFA in rodents [40]. The increased recruitment of inflammatory cells to the inflamed joint, is known to be the major source of these oxidant molecules in patients with RA [2,15]. Specifically, the activity of the inflammatory cells in the inflamed joint leads to release of cytotoxic metabolites such as ROS and RNS that result in bone deformity seen in patients with RA [2,43–45]. Moreover, increased levels of malondialdehyde, a major biomarker of oxidative stress and low levels of endogenous antioxidants have been found in patients with rheumatoid arthritis [2,47]. Low levels of GSH, tocopherols, β -carotene and SOD are known to be

associated with RA [48]. Thus, the use of antioxidants is being proposed as a strategy for the prevention or treatment of this chronic inflammatory disorder [2,47–51]. The results of this study showed that JB demonstrated potent antioxidant effect, as it reduced the levels of MDA and nitrite; accompanied by increased antioxidant molecules (GSH and SOD) in the joint tissue of CFA-mice. This finding, which collaborates the reports of Benson et al. [26] further suggest that JB supplementation may serve as an option for the prevention of free radical-mediated tissue injury associated with chronic inflammatory diseases.

Both preclinical and clinical studies have shown that RA does not only affect the physical activity of the sufferers but also impair the psychosocial wellbeing of the individuals [2,52,53]. RA like every chronic disease is known to produce psychosocial stress that could precipitate depression and other neurobehavioural deficits such as altered locomotion and postural instability or impaired sensorimotor functions [1,52]. The intense persistent pain due to RA [16] is known to cause a wide range of emotional reactions such as worry, distress, aggression, anger, depression and social withdrawal [1,52,53]. Notably, emotional disturbances related to anxiety, worry or depression also exacerbate painful conditions [61]. Thus, anxiety and depression are integral components of chronic pains in clinical settings [59,60,62]. Previous studies have shown that CFA produced nociceptive responses via the release of pain mediators such as bradykinin, serotonin and prostaglandins [54–56], which either stimulate or sensitize nociceptors to cause pain in response to algogenic substances [57,58]. However, JB was found to slightly elevate the pain threshold to a mechanical stimulus in the inflamed hind paw of CFA-mice. Meanwhile, apigenin and naringenin, which are major constituents of JB have been reported to exhibit analgesic activity in both acute and chronic animal models of pain [30,67]. These findings appear to support the acclaimed benefit of JB in patients with arthritic pains in ethnomedicine.

In the neurobehavioural studies, JB was found to reverse motor dysfunctions and also reduced anxiety and depressive-like behaviours induced by CFA in mice. It is generally believed that RA alters physical activity (PA) of the patients as a consequence of joint pain and fatigue [5]. Previous studies using accelerometer have shown that PA was lower in RA patients compared with the general population, though only in terms of moderate and vigorous activity [5]. An accelerometer like an activity cage is known to capture salient behavioural characteristics including the frequency, intensity and duration of PA, as well as resting periods [5]. However, the results of this study showed that CFA slightly increased activity counts but significantly impaired sensorimotor functions, as revealed by reduced locomotion, increased falls and decreased number of turnings in the walk beam test. The slight increase in activity counts in CFA-mice might have resulted from overall increase in bodily activity occasioned by severe pain despite reduced active movement. However, the findings that JB attenuated the sensorimotor deficits induced by CFA suggest that it might be helpful in improving the quality of life of arthritic patients. This assertion is further confirmed from the findings that JB attenuates anxiety- and depression-like behaviours induced by CFA in mice. Previous studies have established heightened anxiety and depression as the major medical conditions that affect the psychosocial functioning of patients suffering from RA [63–65]. Moreover, CFA-induced anxiety- and depressive-like symptoms in rodents have been shown to be related to increased oxidative stress in the brain of rodents [40]. In addition, a close association between oxidative stress and depressive illnesses has also been established in literature [13,66]. However, more studies are needed to confirm whether the antioxidant activity of JB has any role in its ability to reduce CFA-induced anxiety and depressive-like symptoms in mice. Meanwhile, Jobelyn® has been shown to possess various bioactive substances such as apigenin, luteolin and naringenin, which have been reported to exhibit antidepressant, antineuroinflammatory, antioxidant and membrane stabilizing properties [23,26–28]. However, the roles of these active constituents in the anti-arthritis effect of JB remain to be investigated as future studies.

5. Conclusions

The results of this study show that JB attenuates inflammatory and nociceptive responses as well as neurobehavioural deficits induced by CFA in mice, which support its acclaimed benefit in individuals with arthritic disorder. The attenuation of complete Freund-adjuvant-induced inflammatory responses may involve inhibition of oxidative stress and release of proinflammatory cytokines.

Conflict of interest

The authors declare that they have no conflict of interest.

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