Jobelyn® exhibited anti-inflammatory, antioxidant, and membrane-stabilizing activities in experimental models

Abstract

**Background:** Jobelyn® (JB) is an African sorghum-based food supplement claimed to be efficacious for the treatment of rheumatoid arthritis (RA). Although in vitro studies confirmed its anti-inflammatory property, no study had shown the effect of JB using in vivo animal models of inflammation. Thus, its effects on acute and chronic inflammation in rats were evaluated in this study. Its effect on rat red blood cell (RBC) lysis was also assessed.

**Methods:** Acute inflammation was induced with intraplantar injection of carrageenan and increase in rat paw volume was measured using plethysmometer. The volume of fluid exudates, number of leukocytes, concentrations of malondialdehyde (MDA), and glutathione (GSH) in the fluid were measured on day 5 after induction of chronic inflammation with carrageenan in the granuloma air pouch model. RBC lysis induced by hypotonic medium as determined by release of hemoglobin was measured spectrophotometrically.

**Results:** JB (50–200 mg/kg) given orally produced a significant inhibition of acute inflammation induced by carrageenan in rats. It reduced the volume and number of leukocytes in inflammatory fluid in the granuloma air pouch model of chronic inflammation. It further decreased the levels of MDA in the fluid suggesting antioxidant property. JB elevated the concentrations of GSH in inflammatory exudates indicating free radical scavenging activity. It also significantly inhibited RBC lysis caused by hypotonic medium, suggesting membrane-stabilizing property.

**Conclusions:** JB has in vivo anti-inflammatory activity, which may be related to its antioxidant and membrane-stabilizing properties, supporting its use for the treatment of arthritic disorder.

**Keywords:** anti-inflammatory; antioxidant; granulomatous inflammation; Jobelyn®; membrane-stabilizing.

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**Introduction**

Rheumatoid arthritis (RA) is a chronic inflammatory disease responsible for the impairment of the quality of life of many people globally. RA is one of the most frequent causes of disability among adults and is second to ischemic heart disease as a cause of work disability in people over 50 years old [1]. While the pathology of RA appears to be multifactorial and mediated by multiple molecular entities, increased activity of inflammatory cells (WBC) appears to play a prominent role in the pathogenesis of the disease [2]. The initiation and progression of the disease are related to the migration of WBC to the inflamed joint in response to the release of inflammatory mediators such as cytokines, prostaglandins, and leukotrienes [3–6]. The activity of WBC results in the release of free radicals and other cytotoxic products, which initiate and propagate bone destruction seen in patients with RA [4–8].

The progressive deterioration of articular cartilage in RA results in intense pain and difficulty with physical activities. The intense persistent excruciating pains that characterize RA necessitates prolonged palliative medications focusing on the alleviation of symptoms as cure for the disease still remains elusive [2]. Current therapies focus on the alleviation of symptoms with the use of
nonsteroidal anti-inflammatory drugs (NSAIDs) and corticosteroids but these drugs cannot alter the course of the disease pathology. In addition, their usefulness is often compromised by high risks of toxicity [2, 3]. As alternatives to these conventional drugs, a number of medicinal plants with multiple sites of action that could target the various phases of the inflammatory pathways might serve as new medicines for RA and joint health [1].

Previous investigations had reported improvements in arthritic pain with the use of new molecules of plant origins [1, 9]. The efficacy of these agents in RA was ascribed to inhibition of both WBC-mediated free radical generation and the release of inflammatory mediators [1, 2, 10]. Jobelyn® (JB), a sorghum-based nutritional supplement derived from the polyphenol-rich leaf sheathes contains several active ingredients with potent antioxidant activity [11]. In addition to its use as a remedy for anemia, JB has also gained international recognition for the relief of arthritic pain and to promote good joint health [11]. A recent study carried out in culture cells shows that JB inhibited infiltrations of WBC, release of inflammatory mediators, and formation of free radicals [12], which are the major culprits involved in the pathological abnormality of RA [2, 5, 7]. In addition, Benson et al. [12] reported that JB prevented cell membrane damage and inhibited the formation of free radicals, which usually trigger the release of inflammatory cytokines. Also, previous studies have shown that several phytochemicals such as luteolin, naringenin, and apigenin found in JB demonstrated anti-inflammation in culture cells [13, 14]. Luteolin, in particular, was shown to inhibit nuclear factor-κB (NF-κB) signaling in immune cells, which supports its therapeutic efficacy in conditions associated with chronic inflammation [11]. However, no studies have been carried out to confirm its efficacy using in vivo animal models of inflammation that closely mimic the pathology of RA. This study was therefore designed to evaluate the in vivo anti-inflammatory activity of JB on acute and chronic inflammatory models in rats. Its effect on membrane stabilization was also assessed in the study based on inhibition of rat red blood cell (RBC) lysis induced by hypotonic medium.

Materials and methods

Laboratory animals

Male Wistar rat (120–200 g) used for this study were obtained from the Central Animal House University of Ibadan. They were kept under standard environmental conditions and they had free access to commercial food pellets and water ad libitum. They were acclimatized for 2 weeks before commencement of the experiment. All procedures in this study were performed in compliance with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving the Care and Use of Laboratory Animals.

Drugs and chemicals

Jobelyn® (Health Forever Products Ltd, Lagos, Nigeria), indomethacin (Sigma Aldrich, USA), carrageenan (Sigma Aldrich, USA), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB; Sigma Aldrich, USA), trichloroacetic acid (Sigma Aldrich, USA), thiobarbituric acid (Sigma Aldrich, USA), sodium carbonate (BDH Poole, England), potassium carbonate (BDH Poole, England), and sodium chloride (BDH Poole, England) were used in this study.

Effect of JB on carrageenan-induced paw edema

Effect of JB on acute inflammation induced by carrageenan was evaluated according to the method previously described [15]. The animals were divided into five treatment groups consisting of six animals per group. The first three groups were treated orally with JB (50, 100, or 200 mg/kg), whereas the 4th and the 5th groups received indomethacin (10 mg/kg) and distilled water (10 mL/kg), respectively. One hour after the treatment, the right hind paw volume of each rat were determined before induction of acute inflammation with subplantar injection of 0.1 mL of 1% carrageenan [15]. The increase in paw volume was measured at the 3 h using Ugo Basile plethysmometer (Comerio VA, Italy). The increase in paw volume and percentage inhibition of inflammatory edema were calculated as previously described [15].

Membrane-stabilizing activity of JB

Membrane-stabilizing activity of JB was evaluated using inhibition of rat RBC hemolysis induced by hypotonic medium according to the method previously described by Shinde et al. [10]. Blood was collected by cardiac puncture from male Wistar rats under ether anesthesia. The blood was mixed with equal volume of sterilized Alsever’s solution and was then centrifuged at 3000 g for 10 min using Uniscope Laboratory Centrifuge (made in China). The packed cells were washed with sodium phosphate saline buffer (0.1 M, pH 7.2) three times and a suspension in 10% PBS was made. The RBC suspension (0.5 mL) was incubated with 5 mL of either hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) or isotonic-buffered solution of JB (0.25, 0.5, 1.0, and 2.0 mg/mL) or indomethacin (0.5 mg/mL). The control sample consisted of 0.5 mL of RBC mixed with hypotonic-buffered saline solution alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm using spectrophotometer (Inesa, made in China). The percentage inhibition of hemolysis or membrane stabilization was calculated as described by Shinde et al. [10].
of Selye [16] as described by Boris and Stevenson [17]. Twenty millimeters of air, followed by 2 mL of 100 mg/kg of carrageenan in 1.0 mL of groundnut oil was injected subcutaneously into the shaved dorso-lateral skin surface of the animals. The animals (six rats per group) were treated daily with JB (50, 100, or 200 mg/kg), indomethacin (10 mg/kg), or distilled water (10 mL/kg) orally for 5 consecutive days, beginning on day 1 prior to the administration of carrageenan suspension. On the 5th day, the animals were sacrificed under ether anesthesia. The inflammatory exudates obtained were measured using a graduated cylinder. On the 5th day, the animals were sacrificed under ether anesthesia. The inflammatory exudates obtained were measured using a graduated cylinder.

Biochemical assays

**Effect of JB on the levels of glutathione in inflammatory fluid exudates:** Glutathione (GSH) is a potent endogenous antioxidant molecule that protects cells against the damaging effect of free radicals by scavenging them [18]. We estimated the levels of GSH in the inflammatory exudates using a similar procedure described by Moron et al. [19]. Equal volume (0.4 mL) of exudates and 20% trichloroacetic acid (TCA) (0.4 mL) were mixed. The mixture was 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. 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 using a spectrophotometer (Inesa, made in China).

**Effect of JB on the concentrations of malondialdehyde in fluid exudates:** Effect of JB on the concentrations of malondialdehyde (MDA), a measure of the extent of lipid peroxidation was determined according to the method by Okhawa et al. [20]. A mixture of 0.5 mL of distilled water and 1.0 mL 10% trichloroacetic acid was added to 0.5 mL of the fluid exudates. The mixture was then centrifuged at 3000 g for 10 min and 0.1 mL of thioarbituric acid (0.375%) was added to 0.9 mL of the supernatant. The mixture was then incubated in a water bath (Equitron, Mumbai, India) at 80 °C for 40 min. Upon cooling, the absorbance of the supernatants was read at 532 nm using a spectrophotometer (Inesa, made in China). The concentration of MDA in the inflammatory exudates was expressed as micromoles per mL (μmol/mL).

**Estimation of leukocyte counts in inflammatory fluid exudates**

The number of leukocytes in the inflammatory exudates was estimated according to the procedure previously described by Higgs et al. [21]. The inflammatory exudates (0.05 mL) were mixed with 2% acetic acid and 1% methylene blue. After thorough mixing, two drops of the mixture (dilution) were filled on both sides of improved Neubauer counting chamber using a pipette. Then the Neubauer counting chamber (Marienfeld laboratory Glassware, made in Germany) was placed in Petri dish for 3 min to allow the cells to settle. After the cells have settled on the hemacytometer (Marienfeld laboratory Glassware, made in Germany), it was then placed under the light microscope (Olympus optical, made in Japan) (40×) and the number of leukocytes was estimated. The mean leukocyte count per mL of inflammatory exudates for each tested group was expressed as a percentage of the control value [21].

**Histological examination**

Histological examination of the pouch tissue lining was done to further show the protective effect of JB against granulomatous inflammation caused by carrageenan in rats. The pouch tissues of the rats were dissected, washed in distilled water, and fixed in 10% neutral buffered formalin. The tissues were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The tissue lining was later viewed using light microscope (Olympus optical, made in Japan) to detect the severity of tissue damage.

**Statistical analysis**

Data were expressed as the mean±SEM. Data were statistically analyzed using a one-way analysis of variance (ANOVA, Graph Pad Prism 5.0), followed by Bonferroni multiple comparison tests. Statistical significance was determined at a level of p<0.05.

**Results**

**Jobelyn® reduces inflammatory edema induced by carrageenan in rats**

Intraplantar injection of carrageenan characteristically produced a significant increase in paw edema volume when compared with control (Table 1). As shown in Table 1, JB (100 and 200 mg/kg, p.o.) produced a significant (p<0.05) inhibition of inflammatory edema induced by carrageenan (1%) in rats in comparison with control. Similar effect was observed in animals treated with indomethacin (10 mg/kg p.o.), a standard anti-inflammatory drug (Table 1).

**Jobelyn® inhibited rat RBC lysis induced by hypotonic medium**

As shown in Table 2, JB (0.5, 1.0, and 2.0 mg/mL) produced a significant inhibition of rat RBC lysis induced by

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose, mg/kg</th>
<th>Mean change in paw volume</th>
<th>Inhibition of paw edema, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>1.61±0.21</td>
<td>–</td>
</tr>
<tr>
<td>Jobelyn®</td>
<td>50</td>
<td>1.24±0.11</td>
<td>23.3</td>
</tr>
<tr>
<td>Jobelyn®</td>
<td>100</td>
<td>0.90±0.13*</td>
<td>43.8</td>
</tr>
<tr>
<td>Jobelyn®</td>
<td>200</td>
<td>0.64±0.15*</td>
<td>60.4</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>10</td>
<td>0.76±0.16*</td>
<td>52.5</td>
</tr>
</tbody>
</table>

Each value represents the mean±SEM for five animals in each group, *p<0.05 when compared to distilled water-control group (ANOVA and Bonferroni multiple comparison tests).
Umukoro et al.: In vivo anti-inflammatory activity of Jobelyn®

Table 2: Protective effect of Jobelyn® against hypotonic medium-induced rat red blood cell hemolysis.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Concentration, mg/mL</th>
<th>Optical density</th>
<th>Inhibition of RBC lysis, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>0.12±0.00</td>
<td>–</td>
</tr>
<tr>
<td>Jobelyn®</td>
<td>2.0</td>
<td>0.03±0.01*</td>
<td>75</td>
</tr>
<tr>
<td>Jobelyn®</td>
<td>1.0</td>
<td>0.03±0.00*</td>
<td>75</td>
</tr>
<tr>
<td>Jobelyn®</td>
<td>0.5</td>
<td>0.04±0.00*</td>
<td>66.6</td>
</tr>
<tr>
<td>Jobelyn®</td>
<td>0.25</td>
<td>0.08±0.00*</td>
<td>67</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.5</td>
<td>0.05±0.00*</td>
<td>58.3</td>
</tr>
</tbody>
</table>

Each value represents the mean±SEM for three sets of experiment. *p<0.05 when compared with control (ANOVA and Bonferroni multiple comparison tests).

Effect of Jobelyn® on the volume of inflammatory exudates in rats

The effect of JB on the volume of inflammatory fluid produced by carrageenan in the granuloma air pouch model of chronic inflammation is shown in Figure 1. As shown in Figure 1, JB (100 and 200 mg/kg) produced a significant suppression of the volume of inflammatory fluid formation when compared with control. In a similar manner, indomethacin (10 mg/kg) also reduced the volume of fluid exudates evoked by carrageenan in rats. However, JB (50 mg/kg) did not produce a significant decrease in the volume of fluid exudates in comparison with control (Figure 1).

Jobelyn® reduces leukocyte counts in inflammatory exudates

The effect of JB on infiltrations of leukocytes to the site of inflammatory injury in the granulomatous inflammation is presented in Figure 2. As shown in Figure 2, JB (100 and 200 mg/kg, p.o.) exhibited a significant (p<0.05) decrease in the number of leukocyte counts in the inflammatory

![Figure 1: Effect of Jobelyn® on the volume of exudates formation in the granuloma air pouch model of chronic inflammation. Each column represents mean±SEM for six animals. *p<0.05 significant when compared with control (ANOVA and Bonferroni multiple comparison tests). *, **, and *** simply indicate strength of significance.](image1.png)

![Figure 2: Effect of Jobelyn® on leukocytes counts in granuloma air pouch model of chronic inflammation. Each column represents mean±SEM for six animals. *p<0.05 was significant when compared with control (ANOVA and Bonferroni multiple comparison tests). *, **, and *** simply indicate strength of significance.](image2.png)

![Figure 3: Effect of Jobelyn® on the concentrations of malondialdehyde in inflammatory exudates in rats. Each column represents mean±SEM for six animals. *p<0.05 was significant when compared with control (ANOVA followed by Bonferroni multiple comparison tests). *, **, and *** simply indicate strength of significance.](image3.png)
exudates in comparison with control. Indomethacin (10 mg/kg), given orally, also inhibited the infiltration of leukocytes to the site of inflammation in a significant (p<0.05) manner.

**Jobelyn® reduces the concentrations of MDA in inflammatory exudates**

The effect of JB on the concentrations of MDA in inflammatory exudates induced by carrageenan in the granuloma air pouch model of chronic inflammation is shown in Figure 3. The increased concentration of MDA in the inflammatory fluid was significantly (p<0.05) inhibited by JB (50, 100, and 200 mg/kg) in a dose-dependent manner, which indicates antioxidant property. Indomethacin (10 mg/kg) also produced a significant decrease in the concentrations of MDA in the inflammatory exudates in comparison with control (Figure 3).

**Jobelyn® elevated the concentrations of GSH in inflammatory fluid in rats**

Figure 4 shows the effect of JB on the concentrations of GSH in inflammatory exudates induced by carrageenan in the granuloma air pouch model of chronic inflammation in rats. JB (50, 100, and 200 mg/kg, p.o.) demonstrated a significant (p<0.05) elevation in the concentrations of GSH in the inflammatory exudates in comparison with control suggesting free radicals scavenging property. As shown in Figure 4, indomethacin (10 mg/kg, p.o.) significantly increased the concentrations of GSH in the inflammatory exudates.

**Jobelyn® prevented destruction of pouch tissue linings in rats**

Histological examination of the pouch tissues revealed that JB (50, 100, and 200 mg/kg, p.o.) offered a significant (p<0.05) protection against tissue destruction induced by carrageenan in the granuloma air pouch model of chronic inflammation in rats. JB (200 mg/kg, p.o.) prevented tissue destruction and also reduced infiltrations of inflammatory cells in comparison with control (Figures 5 and 6). As shown in Figure 7, indomethacin (10 mg/kg, p.o.) also reduced the severity of tissue damage when compared with control.

**Discussion**

The results of this study revealed that JB inhibited acute inflammation as it significantly reduced edema paw volume induced by carrageenan in rats. Acute inflammation induced by carrageenan is known to be mediated through a step-wise release of chemical mediators such as histamine, bradykinin, and serotonin in the early phase and prostaglandins in the late phase [22]. These mediators caused inflammation through the induction of vasodilatation and increased vascular permeability that enhanced accumulation of fluid in the interstitial tissue [22]. The plasma extravasation that occurs due to contraction of the vascular endothelial cells paves way for fluid exudation and invasion of leukocytes at the site of inflammation [22]. However, prostaglandins are known to be weak in producing inflammation but are better at potentiating the effects of other mediators [23]. Thus, their appearance during the late phase of carrageenan-induced edema further confirms that they are better in enhancing and amplifying the inflammatory effect of earlier released mediators [24]. Inhibition of prostaglandins biosynthesis therefore serves as an important target for development of drugs with anti-inflammatory property. Indeed, NSAIDs owe their clinical efficacy in the treatment of inflammatory conditions to inhibition of prostaglandins synthesis [24, 25]. The findings that JB reduced the paw edema size produced by
carrageenan in rats suggest the presence of phytochemical (s) with anti-inflammatory property.

The anti-inflammatory activity of JB was further evaluated in this study using granuloma air pouch model of chronic inflammation based on the volume of fluid exudates, number of WBC, free radical activity, and pouch tissue histology. JB was found to suppress the volume of fluid exudates and reduce the number of WBC in the inflammatory exudates. In addition, JB produced a significant reduction in the concentrations of MDA and elevation of the levels of GSH in the fluid. Histological examination of the pouch tissues showed that JB offered a significant protection against tissue destruction that characterized the granulomatous inflammation in rats.

Granuloma air pouch model of chronic inflammation is a suitable animal paradigm that closely mimics the pathology of RA as it has many morphological features similar to the disease, including patterns of tissue destructions, infiltration of inflammatory cells, and progression of the disease [8, 24, 26]. The model also shares many of the inflammatory mediators and increased activity of ROS in the synovium and cartilage of patients with RA [2]. Although RA is a disease of multiple pathologies, inflammatory cells are the primary initiators of this disorder as they respond to the deposits of crystals in the joints [2, 27]. In a similar manner, the inflammatory cells respond to the deposit of carrageenan in the granuloma air pouch model of chronic inflammation and during the process of phagocytosis.

Figure 5: Photomicrograph of the tissue pouch lining in distilled water-control group during granulomatous inflammation in rats. The white arrow shows the epidermis layer with moderate infiltration of inflammatory cells. The blue arrows show the dermis layer with diffused inflammatory cells and the attached muscles which have undergone atrophy.

Figure 6: Photomicrograph of the effect of Jobelyn® (200 mg/kg) on the tissue pouch lining during granulomatous inflammation in rats. The white and black arrows show normal epidermis and dermis layers, respectively. The blue arrow shows normal sebaceous glands. The slender arrow shows normal stratum corneum of the epidermis consisting of keratin but with mild infiltration of inflammatory cells.
Inflammatory mediators such as cytokines, prostaglandins, and leukotrienes are released [24, 28]. These mediators in turn produce inflammation and further promote the infiltration of WBC to the site of inflammation [5]. The activity of WBC also results in the release of free radicals, which in turn initiate and propagate tissue and bone destruction that characterize granulomatous inflammation and RA, respectively [5–7]. Thus, an effective therapy for the treatment of the disease should be directed at inhibition of WBC migration and WBC-mediated release of free radicals and other cytotoxic substances. The findings that JB significantly modified the components of granuloma air pouch model of chronic inflammation assessed in this study suggest that it may play a role in the management of chronic inflammatory disorders such as RA. Inhibition of RBC lysis observed in this study further confirms the ability of JB to protect biological membranes such as lysosomal membrane against injurious stimuli that are involved in the initiation and propagation of chronic inflammation. Previous studies have shown that prevention of RBC lysis is a biochemical index for in vitro anti-inflammatory property, especially as membrane stabilizers are capable of preventing the release of lysosomal phospholipases that are primary initiators of the inflammatory responses [24, 28–30].

Although more studies are necessary before commenting on how JB exerts its anti-inflammatory activity, these present data suggest inhibition of WBC-mediated release of free radicals and other inflammatory mediators. In addition, the membrane-stabilizing effect shown by JB may have contributed significantly to its anti-inflammatory property. These suggestions are in agreement with the previous in vitro studies which showed that JB inhibited the activity of inflammatory cells in culture cells [12]. These authors also showed that JB demonstrated potent antioxidant activity in vitro assays. The in vitro anti-inflammatory activity of JB was ascribed to the stabilization of lysosomal membrane and inhibition of the release of free radicals and other cytotoxic products [12]. This may also explain the in vivo anti-inflammatory activity shown by JB in this study. Although several phytochemicals such as luteolin, naringenin, and apigenin have been shown to exhibit anti-inflammatory activity in culture cells [13, 14], it remains to be established which of these active principles are involved in the mediation of the anti-inflammatory effect of JB observed in this study.

Conclusions

The results of the study showed that JB has in vivo anti-inflammatory effect both in acute and chronic animal models of inflammation in rats, supporting its use for the treatment of arthritic disorder. This effect may be related to inhibition of infiltrations of WBC as well as its antioxidant and membrane-stabilizing properties.

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