

Bixa orellana Leaves Extract Inhibits Bradykinin-Induced Inflammation through Suppression of Nitric Oxide Production

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Key Words

Anti-inflammatory · *Bixa orellana* · Bradykinin · Vascular permeability

Abstract

Objective: The present study was conducted to assess the anti-inflammatory effect of a crude aqueous extract of *Bixa orellana* leaves (AEBO) and to examine the possible involvement of nitric oxide (NO) in its anti-inflammatory mechanism. **Materials and Methods:** The air-dried, powdered leaves were soaked in distilled water (1:20 w/v) at 50°C for 24 h and the supernatant obtained was freeze-dried (yield 8.5% w/w). The dosage was recorded as the mass of extract per kg b.w. of rats in all inflammatory assays (bradykinin-induced paw edema, peritoneal vascular permeability and NO assay). **Results:** Pretreatment with AEBO for 4 consecutive days exhibited significant inhibitory activity against inflammatory models, the bradykinin-induced hind paw edema model and bradykinin-induced increased peritoneal vascular permeability at both doses in dose-dependent manner. In addition, AEBO was also found to significantly suppress the production of NO at doses of 50 and 150 mg/kg. **Conclusion:** This study provides scientific data to support the tradi-

tional use of *B. orellana* leaves in treating inflammation. Results from this study suggest that AEBO exerts anti-inflammatory effects. Part of this anti-inflammatory effect may be associated with its antibradykinin activity and may be related to a reduction of the NO production.

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Introduction

There are a number of inflammatory mediators released by cells in response to localized injury and trauma. Bradykinin, one of the peptide kinins, is generated in plasma and in a variety of peripheral tissues in response to mechanical injury or infection. It is an important inflammatory mediator, as well as an endothelium-dependent vasodilator, involved in both the initiation and progression of an inflammatory response where it sensitizes nociceptor peripheral terminals, reducing the pain threshold [1], promotes vasodilatation [2] and increases the permeability of blood vessels to the plasma component [3]. The pro-inflammatory effects of bradykinin are mediated by at least 2 receptors: the B1 receptor is undetectable and usually induced following tissue inflamma-

tion and damage; on the other hand, the B2 subtype is expressed constitutively and mediates many of the actions of bradykinin [4].

Bixa orellana L or annatto, from the family Bixaceae, is native to Central and South America [5] and often cultivated in countries like Brazil, Peru, Kenya, India, China, the Philippines and Malaysia. The native people of Malaysia drink a decoction of its leaves as a remedy for gastric ulcers and stomach discomforts. In Peruvian medicine, the leaves are used to treat several disorders including prostate disorders and internal inflammation [6, 7]. The leaves are also boiled to treat colic, as well as oral and throat inflammation [6, 8, 9]. It is also one of the plants used by Rama midwives in Nicaragua for the treatment of indigestion and other digestive disorders [10] and in Guinean traditional medicine to treat infectious diseases [11].

Previous studies reported the presence of leucocyanidin, ellagic acid, apogenin, luteolin, and flavonoid bisulphates [12], while Lawrence and Hogg [13] reported the presence of ishwarane and bixaghanen in *B. orellana* leaf oil. The pharmacological action of its leaves' extract includes anticonvulsant, analgesic, antidiarrheal [14], antimicrobial [15], antileishmanial and antifungal activity [16]. In a previous study, Siti Erli Fazira et al. [17] showed that *B. orellana* plays an important role in anti-inflammatory responses induced by carrageenan. *B. orellana* is also able to neutralize the edema-forming effects of *Bothrops asper* venom [18]. In order to provide scientific support for some of these claims, the present study aimed to evaluate the anti-inflammatory effect of *Bixa orellana* leaves using standard experimental models. Aqueous extract of *B. orellana* (AEBO) was used to treat inflammation in animal models and the possible involvement of nitric oxide (NO) in its anti-inflammatory mechanism was examined.

Materials and Methods

Experimental Animals

The experiments were carried out using male Sprague-Dawley rats (200–250 g) housed at the animal house in the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia. The animals were maintained under standard laboratory conditions with access to food and water ad libitum. They were allowed a 7-day acclimatization period to adjust to the conditions. The rats were randomly selected and assigned into 4 different treatment groups of 6 rats each. Two groups of rats received AEBO at doses of 50 and 150 mg/kg, while the positive group received D-Arg-L-Arg-L-pro-L-Hyp-Gly-L-(2-thienyl)Ala-L-Ser-D-1,2,3,4-tetrahydro-3-isoquinolinecarbonyl-L-(2 α ,3 β ,7 α)-octahydro-1H-indole-2-carbonyl-L-Arg (HOE 140) and the negative group was treated with distilled water only. All experimental pro-

cedures were carried out in strict compliance with the regulations of the Animal Care and Use Committee, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

Chemicals

Bradykinin, Evans Blue Dye and HOE 140 were purchased from Sigma (Malaysia). Nitric/nitrate assay kit was purchased from Roche (Malaysia).

Plant Material

The fresh leaves of *B. orellana* were procured from around the Universiti Putra Malaysia, and were botanically identified and deposited at the Phytomedicinal Herbarium, Institute of Bioscience, Universiti Putra Malaysia (Voucher No. NL16, *Bixa orellana*).

Plant Extraction

The leaves were oven-dried at 60°C for 3 consecutive days and ground into powder-form with a grinder. For the preparation of aqueous extracts, the leaf powder was mixed in distilled water based on the ratio of 1 part powder to 20 parts distilled water (1 g: 20 ml). The mixture was placed in a water bath at 50°C for 24 h in order to allow bioactive compounds in the leaves to dissolve in distilled water. The extracts were filtered and kept at -80°C, then freeze-dried at high vacuum at -40 \pm 5°C (yield 8.5%, w/w). In all experiments, the dosage was recorded as the mass of extract/kg b.w. of rats).

Preliminary Phytochemical Analysis

The AEBO leaves was subjected to a preliminary phytochemical screening for the presence of flavonoids, tannins, anthraquinones and saponins [19].

Anti-Inflammatory Assays

Bradykinin-Induced Paw Edema. The anti-inflammatory assay was performed according to method by Winter et al. [20]. Edema was induced in the left hind paw of the animals by subcutaneous injection of 0.1 ml of bradykinin (0.4 μ g/ml in saline) into the footpad. Edema was indicated from the changes in paw volumes measured using plethysmometer (Model 7140, Ugo Basile, Italy) before and at 30, 60, 120, 180 and 240 min, following bradykinin injection. The AEBO (50 and 150 mg/kg) was given orally for 4 days. Last treatment was given 60 min before bradykinin injection on the fourth day. Another group of rats was orally administered with HOE 140 1 mg/kg, a bradykinin antagonist, as a standard reference. The control group received distilled water.

Bradykinin-Induced NO Production. NO was measured by the method of Moshage et al. [21] using commercially available nitrite/nitrate colorimetric kit (Roche). The sum of nitrites and nitrates was used as indicator of NO level. Following bradykinin injections (0.4 μ g/ml in saline) on the fourth day, inflamed rat paws were homogenized with 35% sulfosalicylic acid and then centrifuged to obtain the supernatant. Assays were carried out on the same day and the concentrations of nitrite and nitrate were measured via spectrophotometry at 540 nm wavelength. A standard curve was constructed using known concentrations of sodium nitrite and potassium nitrate. Values obtained from this procedure represent the NO concentrations in paw tissues, in μ M/g wet weight of tissue.

Bradykinin-Induced Increased Peritoneal Vascular Permeability. The changes of vascular permeability during bradykinin-induced inflammation were studied using Evan's blue dye as a de-

Table 1. Effect of oral administration of AEBO on bradykinin-induced nitric oxide production in rat paw

Treatment	Dose, mg/kg	NO Level, $\mu\text{M/g}$	Inhibition, %
Negative control (distilled water)	-	10.22 ± 0.33	-
HOE 140	1	$7.08 \pm 0.33^*$	30.72
AEBO	50	$8.21 \pm 0.59^*$	19.67
	150	$5.13 \pm 0.57^*$	49.80

Values are mean \pm SEM (n = 6). * p < 0.05 vs. control, 1-way ANOVA.

Table 2. Effect of oral administration of AEBO on bradykinin-induced vascular permeability in 6 rats

Treatment	Dose, mg/kg	Dye leakage, $\mu\text{g/rat}$	Inhibition, %
Negative control (distilled water)	-	30.33 ± 0.87	-
HOE 140	1	$3.38 \pm 0.08^*$	88.87
AEBO	50	$9.07 \pm 0.37^*$	70.08
	150	$5.38 \pm 0.29^*$	82.27

Values are mean \pm SEM. * p < 0.05 vs. control, 1-way ANOVA.

tector [22]. This study was based on spectrophotometric measurements of the dye that was present in peritoneal fluid indicating vascular leakage. One hour after oral administration of AEBO (50 and 150 mg/kg), rats were injected with 5 ml/kg of 1% Evans blue solution intravenously followed by bradykinin intraperitoneally. HOE 140 (1 mg/kg) served as a reference drug, while animals in the control group received an equal volume of saline (10 ml/kg). The rats were sacrificed 30 min after Evan's blue injection, and its extravasations into the peritoneal cavity were measured via spectrophotometry at 610 nm wavelength.

Statistical Analysis

All the results were expressed as mean \pm SEM. Data were analyzed using 1-way ANOVA followed by Tukey test. p < 0.05 was considered as statistically significant.

Results

Preliminary Phytochemical Data

Results of the preliminary phytochemical analysis carried out on the crude aqueous extract indicated the presence of flavonoid, tannins, anthraquinones and saponins.

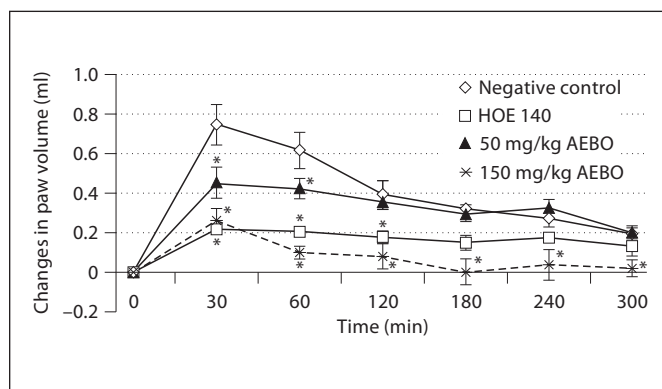


Fig. 1. Effect of aqueous extract of *Bixa orellana* leaves on bradykinin-induced hind paw edema in rats. * p < 0.05 vs. control at each time point.

Effect on Bradykinin-Induced Paw Edema

Rat paws became edematous soon after injection of bradykinin and paw volume reached a maximum after 30 min (fig. 1). AEBO significantly suppressed the edema formation in a dose-dependent manner. At doses of 50 and 150 mg/kg, AEBO inhibited paw swelling by 39.9 and 65.6% compared to control values at 30 min post-injection of bradykinin, respectively. Post-hoc analysis showed significant inhibition of edema formation at the highest dose of AEBO at all time points, whereas at 50 mg/kg, AEBO was effective at 30 and 60 min time points, respectively.

Effect on Bradykinin-Induced NO Production

After treatment with bradykinin, the NO concentration increased markedly to $10.22 \pm 0.33 \mu\text{M}\cdot\text{g}^{-1}$ (p < 0.05). When rats were treated with 50 and 150 mg/kg of AEBO, NO production was significantly suppressed in a dose-dependent manner ($8.21 \pm 0.59 \mu\text{M}\cdot\text{g}^{-1}$, $5.13 \pm 0.57 \mu\text{M}\cdot\text{g}^{-1}$, respectively, p < 0.05). Percentages of inhibition at both doses were 19.7 and 49.8%, respectively (table 1).

Effect on Bradykinin-Induced Increased Peritoneal Vascular Permeability

At doses of 50 and 150 mg/kg, AEBO exerted a significant effect against acute inflammation induced by bradykinin (table 2) and the inhibition of dye leakage was 70.1 and 82.3%, respectively. As a positive control, HOE 140 produced a more potent anti-inflammatory effect, 88.9% inhibition of dye leakage.

Discussion

The present study showed that AEBO, when administered orally, possessed anti-inflammatory activity. The result obtained from this study showed that at oral doses of 50 and 150 mg/kg, AEBO inhibited the paw edema induced in rats by bradykinin at 30 min post-induction. It is well known that both tannins and flavonoids have anti-inflammatory properties [23]. Thus, the anti-inflammatory activity inherent in AEBO may be related to the presence of tannins and flavonoids in the AEBO.

In the first stage of inflammatory reactions, mediators of inflammation are released following stimulation, leading to dilation of arterioles and venules and increased vascular permeability [24]. The present results also show the ability of AEBO to reduce the leakage of Evan's Blue dye into the peritoneal space during peritoneal inflammation produced by bradykinin in rats, indicating its ability to inhibit or suppress the permeability of small blood vessels. Studies by Félétou et al. [25] showed that when bradykinin is used as an inflammatory mediator to induce microvascular permeability in the hamster cheek pouch, the microvascular leakage is mediated by the B2 receptor activation but not the B1 receptor. This suggests that B2 receptors play a more important role in raising vascular permeability. The anti-inflammatory activity of AEBO may involve an inhibitory effect on bradykinin receptor or any signaling molecules further downstream to prevent vascular permeability from increasing.

Bradykinin markedly increased microvascular permeability and this action appeared to be related to the

production of NO or a NO-containing compound [26]. Nitric oxide is an important inflammatory mediator and regulatory molecule for various physiological functions, such as neurotransmission, vasodilatation and important for host defense [27]. The NO produced by endothelial nitric oxide synthase mediates vascular relaxation, but when produced in high amounts is able to increase the vascular permeability [28]. The possible mechanism that is involved in vascular permeability suppression by AEBO was investigated through determination of NO level in paw fluid. From the results obtained, we can suggest that AEBO produces its anti-inflammatory activity by preserving the endothelial barrier function of the small vessels, an action largely dependent on the suppression of NO formation. Furthermore, the effect of AEBO was comparable to that of the reference drug, HOE 140 (specific antagonist for B2 receptor) used in this study. It has been shown that HOE 140 could decrease vascular permeability through inhibition of nitrite release [29].

Conclusion

The results of the present study showed that AEBO possesses significant anti-inflammatory activity against the acute phase of inflammation, which may be due to its antibradykinin activity. These results provide support for the traditional use of *B. orellana* leaves in inflammation, but warrant further studies to establish its therapeutic value as well as its mechanism of action.

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