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# Evaluation of Anticolitis and Antioxidant Properties of *Bixa orellana* (Bixaceae) Leaf Hydroethanolic Extract on Acetic Acid-Induced Ulcerative Colitis in Rats

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ABSTRACT

*Background:* Ulcerative colitis is an idiopathic inflammatory bowel disease characterized by tissue damage, diarrhea, anemia, and loss of body weight. Tissue damage occurs as a result of uncontrolled activation of the immune response and an increase in free radicals, which have a strong effect on the pathogenesis of inflammatory bowel disease. The incidence and prevalence of this inflammatory disease continue to increase worldwide. Maceration of *Bixa orellana* leaves in palm wine is used in traditional medicine to treat diarrhea, dysentery, and hemorrhoids in the Adamaoua region of Cameroon.

*Objective:* The present work evaluated the preclinical effects (ie, antioxidant, hematological, and histological activities) of the hydroethanolic extract of *Bixa orellana* leaves in an in vivo, rat acetic acid-induced ulcerative colitis model.

*Methods:* Thirty-six female rats weighing between 165 and 180 g were fasted for 18 hours and then anesthetized with ether. A dose of 1 mL acetic acid (5%) was administered rectally through a catheter in all rats except the normal control group, which received distilled water (1 mL) instead. Treatments began 48 hours after rectal administrations of acetic acid or water, and all animals were treated twice daily for 7 days. The normal control group and the colitis control group received PO distilled water (10 mL/kg), the positive control received orally loperamide (5 mg/kg, and the 3 test groups received orally the hydroethanolic extract of *Bixa orellana* at 100, 200, and 400 mg/kg, respectively. During treatment, the number of diarrheal stools and weight change were assessed. At the end of the treatment, the animals were put to death under ether anesthesia. Blood was collected postmortem for evaluation of hematological and antioxidant parameters. The abdomen was opened via a midline incision and the colon was removed and emptied of all contents to assess histological and antioxidant parameters.

*Results:* During treatment, the number of diarrheal stools was significantly decreased from day 3 in animals treated with 100 ($P < 0.05$), 200 ($P < 0.05$), and 400 ($P < 0.01$) mg/kg extract compared with the colitis control group. The change in body weight of all extract-treated rats decreased significantly from day 3 (–5.55%; $P < 0.05$) to day 8 (–13.80%; $P < 0.01$) compared with the normal control. In the colitis control, this change ranges from –6.15% on day 2 to –15.13% on day 8. Extract treatment with 100,

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200, and 400 mg/kg significantly reduced ($P < 0.05$) the number of lesions and the relative weight of the colon. The levels of red blood cells, neutrophils, and total white blood cells decreased in the colitis control group, whereas treatment with the extract at doses of 100, 200, and 400 mg/kg was associated with a significant increase in these hematological parameters. Catalase and superoxide dismutase activity and glutathione concentrations all increased significantly ($P < 0.01$) in blood and colon in all extract-treated animals, whereas levels of malondialdehyde and nitric oxide were significantly decreased ($P < 0.01$) compared with the colitis control animals.

*Conclusions:* The hydroethanolic extract of *Bixa orellana* leaves had protective effects against acetic acid-induced ulcerative colitis in rats that was associated with inhibited production of free radicals believed to be responsible for oxidative stress, hematological disorders, and tissue damage in this animal model.



## Introduction

Chronic inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis (UC), are conditions characterized by inflammatory lesions of the digestive tract.[1] Colitis is an irritable bowel syndrome or functional colopathy that is associated with multiple symptoms, including abdominal pain, fever, general fatigue, loss of appetite, anemia,[2,3] weight loss, and diarrhea characterized by loose, semiliquid, or watery and bloody stools.[4] The prevalence and incidence of these conditions have increased dramatically over the past 50 years worldwide. In France, the number of cases of Crohn's disease and UC rose, respectively, from 14 to 200 per 100,000 inhabitants and from 15 to 200 per 100,000 inhabitants.[5] IBD are often associated with bowel dysfunction; physical inactivity; unbalanced, low-fiber, high-residue, and heavily spicy diet; and alcohol, tobacco, and coffee use. IBD is associated with an imbalance between antioxidant activity and reactive oxygen species (ROS), which generates oxidative stress and the weakening of the intestinal immune defense system.[6] These diseases reduce the quality of life and work capacity and increase the disability of the world's population.[5] These IBD symptoms can remit spontaneously or be controlled with treatment based on steroidal and nonsteroidal anti-inflammatory drugs, 5-aminosalycilates, corticosteroids, immunomodulators, antitumor necrosis factor antibodies and by surgery in the event of complications or resistance to medical treatment.[3] These medications, although effective, are associated with deleterious effects such as gastrointestinal tract damage and liver and kidney toxicities.[7] Notwithstanding the progress of conventional medicine, the treatment of IBD remains unsatisfactory due to the high cost of conventional treatments, adverse side effects of medications, as well as lack of advanced health infrastructure. More than 70% of patients with IBDs use alternative and complementary medicine.[8] These shortcomings of modern medicine push populations to resort to less-toxic natural products (eg, medicinal plants) used in local traditional medicines.[9]

*Bixa orellana* is a medicinal plant that has antioxidant, anti-inflammatory, antimicrobial, analgesic, and hypoglycemic activities[10] and is used in the treatment of vomiting, wounds, headaches, and diarrhea.[11] In traditional medicine, maceration of *Bixa orellana* leaves in palm wine is used in the treatment of diarrhea, dysentery, and hemorrhoidal attacks in the Adamaoua region of Cameroon.[11] Our first work on antidiarrheal activity showed that a hydroethanolic extract of *Bixa orellana* leaves was more effective at 200 mg/kg body weight against castor oil-induced diarrhea.[11] Several traditional recipes have been shown to be effective in the treatment of inflammatory pathologies.[7] The aim of this preclinical study was to evaluate the antioxidant, hematological, and histological activities of a *Bixa orellana* hydroethanolic extract on acetic acid-induced UC in rats.

## Materials and Methods

### *Plant material and extraction*

*Bixa orellana* leaves were harvested in June 2020 between 8 A.M. and 10 A.M. on the campus of the University of Ngaoundéré (Vina Division, Adamawa Region, Cameroon). A plant sample consisting of stem, leaves, and flowers was collected and then identified by Professor Pierre Marie Mapongmetsem of the Faculty of Science, University of Ngaoundéré, and this specimen was deposited at the Cameroonian National Herbarium under the number 14099/SRF.Cam. The leaves of *Bixa orellana* were washed with tap water, dried in the shade at room temperature and then pulverized. The dried leaves were then crushed and the resulting powder was saved for extraction.

Two hundred grams (200 g) of *Bixa orellana* leaves powder were macerated in 2000 mL ethanol/distilled water (1V/4V) for 72 hours using a stainless steel ladle. The macerate was filtered through Wathman filter paper No. 3, then concentrated in a rotary evaporator and oven dried at 40°C.[11,12] After drying, the extract was weighed and the yield was determined by the following formula:

$$Yield(\%) = \frac{\text{Mass of plant extract (g)}}{\text{Mass of plant powder (g)}} * 100 \quad (1)$$

Forty-three grams of brown extract was obtained, yielding 21.50%. The different extract solutions to be administered to the rats were prepared so that each animal received 1 mL/100 g body weight.[11]

### *Experimental animals*

Thirty-six female Wistar albino rats, aged 9 to 10 weeks and weighing between 165 and 180 g, were used for the experiment. IBDs are more common in women.[13] In vivo experiments were performed in accordance with European Union guidelines for the protection of animals (EEC Council 86/609).[14] The animals were acclimatized for a week in the laboratory before the experiment, where the temperature was approximately 22 ± 2°C with a light/dark cycle of 12/12 hours. The diet consisted of a mixture of corn flour (60%), wheat (10%), fish (12%), soy flour (15%), and palm oil (3%).[15]

### *Induction of colitis and treatment of animals*

Before the start of the experiment, 36 female Wistar albino rats, divided into 6 groups of 6 animals each, were fasted for 18 hours with free access to the water. To induce colitis, all animals were anesthetized with ether and each animal received rectally 1 acetic acid (5%, v/v) solution except for the normal control group, which

received distilled water (1 mL) instead.[16] After administration, the rats were kept in an upright position for 30 seconds to limit reflux of the acetic acid solution or distilled water and to increase the contact time of the acetic acid with the colonic mucosa.[12,17] Forty-eight hours after colitis induction, the animals were treated with *Bixa orellana* hydroethanolic extract, loperamide, or distilled water. Oral administration of the different treatments was performed twice a day (6 A.M. and 6 P.M.) for 7 days by gavage using an esophageal probe as follows:

- Group I (normal control [NC]) received 10 mL/kg distilled water,
- Group II (colitis control [CC]) received 10 mL/kg distilled water,
- Group III (positive control [Lop5]) received 5 mg/kg loperamide (Imodium; Johnson&Johnson, New Brunswick, New Jersey),
- Group IV (Bo100) received 100 mg/kg *Bixa orellana* hydroethanolic extract,
- Group V (Bo200) received 200 mg/kg *Bixa orellana* hydroethanolic extract, and
- Group VI (Bo400) received 400 mg/kg *Bixa orellana* hydroethanolic extract.

During treatment, the quality and number of diarrheal stools and animal behavior were assessed and recorded daily.[17] Similarly, the animals were weighed every morning before administration of the treatment[18] using a precision balance (0.01 g). At the end of treatment, all rats were fasted for 18 hours with free access to water. They were then weighed, anesthetized, and humanely killed by cervical dislocation. Blood samples were collected by cardiac puncture[19] using 10 mL syringes and 21-gauge needles. A total of 2 mL blood was transferred into EDTA tubes for hematological analyses. A sample of 5 mL blood was transferred into dry tubes without additives (BD vacutainer; BD, Franklin Lakes, New Jersey), left in the open air for 10 minutes for coagulation and then centrifuged at 3000 rpm for 15 minutes at 4°C, for analysis of oxidative stress parameters. The colon was removed, emptied of its contents, weighed, and the length measured with a tape measure, which allowed us to determine the linear weight and the relative weight (RW) of colon by the formulas:

$$Linear\ weight\ (mg/mm) = \frac{\text{Mass of emptied colon}}{\text{Colon length}} \quad (2)$$

$$RW\ (\%) = \frac{\text{Organ weight (g)}}{\text{Body weight (g)}} * 100 \quad (3)$$

A sample of 1 cm of sigmoid colon was stored in buffered formalin (10% v/v) solution for 1 week for histological sections. For the preparation of the colon homogenates, 0.5 g of colon was ground using a ceramic mortar and pestle on an ice tray. A total of 2.5 mL Tris buffer was added to each ground material and the mixture was transferred to dry test tubes, then centrifuged at 3000 rpm at 5°C for 25 minutes and the obtained supernatant was collected. The homogenates and sera obtained were stored at −20°C for up to 48 hours for the evaluation of biochemical parameters.

*Assays of biochemical parameters of oxidative stress*

*Assessment of superoxide dismutase activity*

A measure of 0.134 mL of sample fluid (colon homogenates or serum) and 1.800 mL carbonate buffer (0.05 M, pH 10.2) were introduced into the test tubes and the blank tube, respectively. A measure of 1.666 mL carbonate buffer was then added into the test tubes. The reaction was started by adding 0.200 mL adrenaline (0.3 mM) to each tube. The absorbance of the test tubes was measured at 20 and 80 seconds at 480 nm against the blank.[20] The specific activity of superoxide dismutase (SOD) was determined as follows:

$$Inhibition\ (\%) = 100 - \frac{(Ab20s - Ab8Os)test}{(Ab20s - Ab8Os)blank} * 100 \quad (4)$$

Where Ab20s = absorbance measured at 20 seconds and Ab80s = absorbance measured at 80 seconds.

*Assessment of catalase activity*

In the presence of catalase, hydrogen peroxide is broken down and the resulting product binds to potassium dichromate to form an unstable blue-green precipitate of perchloric acid, which is decomposed by heat to form a green complex.[21]

A measure of 50 µL sample fluid (colon homogenates or serum) and 50 µL distilled water were introduced in the test tubes and in the blank tube, respectively. A sample of 750 µL phosphate buffered saline (0.1 mM; pH 7.5) and 200 µL hydrogen peroxide (50 mM) were then added to all the tubes at room temperature for 1 minute and the reaction was stopped by addition of 2000 µL dichromate/glacial acetic acid (5% v/v). The solution was heated at 100°C for 10 minutes and after cooling the absorbance was read at 570 nm against the blank. The specific activity of catalase was determined from the following formula:

$$Catalase\ activity\ (\text{mM H2O2 / min / g}) = \frac{(\text{AbTest} - \text{AbBlank})}{a * t * Ow} \quad (5)$$

Where Ab = absorbance, a = slope of the calibration curve (0.0007), t = reaction time (1 minute), and Ow = organ weight (in grams).

*Determination of colonic and blood glutathione contents*

A measure of 100 µL sample fluid (colon homogenates or serum) and 100 µL Tris-hydrochloric acid buffer (50 mM; pH 7.4) were introduced into the test tubes and the blank tube, respectively. A measure of 1500 µL Ellman's reagent (dinitro-2,2′-dithio-5,5′-dibenzoic acid) was then added to each tube. The mixture was incubated with shaking for 60 minutes at room temperature and the absorbances were read at 412 nm against the blank.[22] The concentration of reduced glutathione (GSH) was determined by the following formula:

$$[GSH]\ (\text{mol / g of organs}) = \frac{(\text{AbTest} - \text{AbBlank})}{(\varepsilon * \text{L} * \text{Ow})} \quad (6)$$

Where Ab = absorbance, L = optical path (1 cm), $\varepsilon$ = molar extinction coefficient (13,600 mol/cm), and Ow = organ weight (in grams).

*Determination of malondialdehyde concentration*

A measure of 125 µL trichloroacetic acid (20% v/v) and 250 µL thiobarbituric acid (0.67% v/v) were added to test tubes containing 250 µL of the colon homogenates or serum and to the blank tube containing 250 µL Tris-hydrochloric acid buffer (50 mM; pH 7.4). All tubes were sealed with glass beads, heated at 90°C in a water bath for 10 minutes, then cooled in tap water and centrifuged at 3000 rpm at room temperature for 15 minutes. The absorbances of the various supernatants of the test tubes were read at 530 nm against the blank.[23] The concentration of malondialdehyde (MDA) was determined by the formula below:

$$[\text{MDA}]\ (\text{mol/g of organs}) = \frac{\boldsymbol{\Delta OD}}{\boldsymbol{\varepsilon} \times \mathbf{L} \times \mathbf{Ow}} \quad (7)$$

Where, MDA = MDA concentration, ΔOD = ODtest − ODblank, L = optical path (1 cm), $\varepsilon$ = molar extinction coefficient (15,600 mol/cm), and Ow: organ weight (in grams).

*Determination of nitric oxide concentration*

A measure of 100 µL colon homogenates or serum diluted in 400 µL distilled water and 500 µL distilled water were introduced respectively into the test tubes and into the blank tube. A measure of 500 µL Griess reagent was then added to each tube. The mixture was homogenized and incubated at room temperature, protected

from light, for 10 minutes, and the absorbance was read at 546 nm against the blank.[24] The concentration of nitric oxide (NO) was calculated using the following formula:

$$[NO] = \frac{\text{ODtest} - \text{ODblank}}{a * Ow} \quad (8)$$

Where NO = nitric oxide concentration, OD = optical density, a = slope of the calibration curve (1.4183), and Ow = organ weight.

*Hematological analyses*

Hematologic, leukocyte, and platelet parameters were evaluated in whole blood using an automatic device (Mindray BC 20s n series TK 65000803, Guangdond China).

*Histological analysis*

Histopathological analyses of colons were carried out by Professor Dzeufiet, Animal Physiology Laboratory of the Faculty of Science of the University of Yaounde I, according to the method described in the literature.[25] The 5-µm thick colon samples were fixed in 10% buffered formalin then dehydrated in ethanol baths of increasing concentration (70%–100%), and then embedded in paraffin. Tissue sections 2-µm thick were made using a microtome, Leica, Germany (Reichert-Jung 2030). These sections were then stained with a hematoxylin/eosin mixture, then examined and filmed under an optical microscope equipped with a camera (MOTIC 1820 LED: SM7432-MC1ST-RPIWFM).

*Statistical analysis*

Statistical analysis of the data obtained was performed using GraphPad Prism version 8.0.1 software (San Diego, California). Data comparison was made using the ANOVA test followed by Tukey's multiple comparison posttest and the differences were considered significant at the 5% level.

## Results

*Animal behavior and appearance of stool*

A few minutes after administration of the acetic acid (5%), the animals remained less mobile, calm, and folded over themselves with erect hairs. The test animals treated with the extract or with loperamide gradually regained their mobility during the treatment. The first bloody and/or mucous diarrheal stools appeared within the third hour after induction of colitis. In normal controls, no diarrheal stool was recorded. In the other groups given acetic acid, the number of diarrheal stools increased significantly ($P < 0.01$) from day 1 to day 7 of treatment in the colitis control and from day 1 to day 3 in rats treated with the extract at 400 mg/kg (Figure 1).

*Effect of Bixa orellana leaves extract on body weight gain*

Twenty-four hours after colitis induction, we observed weight loss in all animals given acetic acid. Normal animals had a gradual increase in body weight throughout the treatment period, whereas in colitis controls and treated animals body weight decreased significantly ($P < 0.01$) during treatment (Figure 2).

*Effect of Bixa orellana leaves extract on colonic ulcerations, linear colon weight, and relative colon weight in rats*

The colon of normal rats showed no ulcerations. The mean (SD) number of lesions was 14.50 (0.52) ($P < 0.01$) in the colitis control group. In the groups treated with loperamide or extracts at

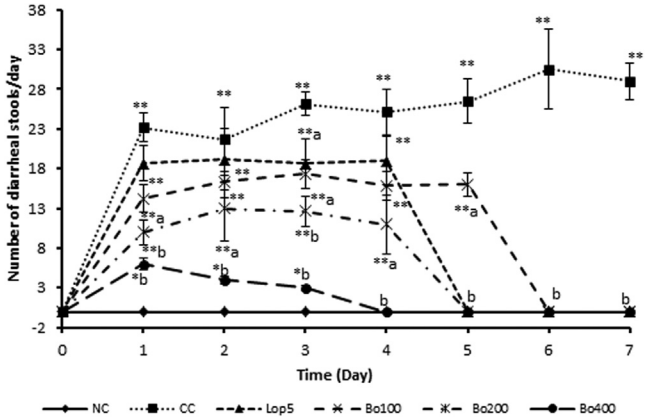


**Figure 1.** Frequencies of diarrheal stools in normal rats (NC), colitis control (CC) group, and rats treated with loperamide (Lop5) and with hydroethanolic extract of *Bixa orellana* at 100 (Bo100), 200 (Bo200), and 400 (Bo400) mg/kg body weight. N = 6. Significant difference: $^{*}P < 0.05$; $^{**}P < 0.01$ between NC and other groups; $^{a}P < 0.05$; $^{b}P < 0.01$ between CC and treated groups.

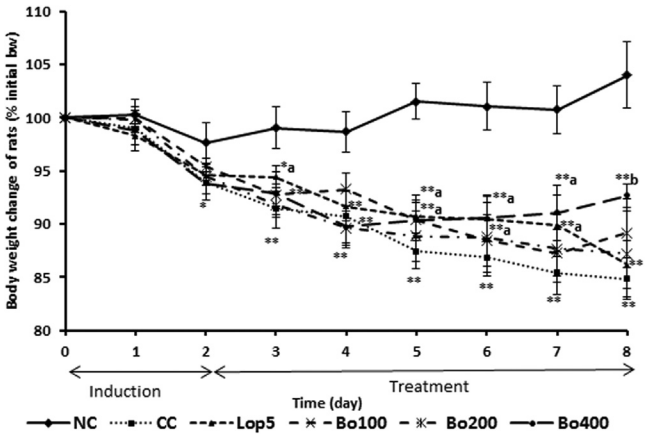


**Figure 2.** Body weight change (%) in normal rats (NC), the colitis control (CC) group, and rats treated with loperamide (Lop5) and with hydroethanolic extract of *Bixa orellana* at 100 (Bo100), 200 (Bo200), and 400 (Bo400) mg/kg body weight. N = 6. Significant difference: $^{*}P < 0.05$; $^{**}P < 0.01$ between NC and other groups; $^{a}P < 0.05$; $^{b}P < 0.01$ between CC and treated groups.

100, 200, and 400 mg/kg body weight, the mean number of lesions were significantly decreased; 8.33 ($P < 0.05$), 3.60 ($P < 0.01$), 5.33 ($P < 0.01$), and 2.4 ($P < 0.01$) respectively in Lop5, Bo100, Bo200, and Bo400 (Figure 3A) The mean linear colon weights were 10.49, 12.82 ($P < 0.05$ vs NC), 10.75, 12.78 ($P < 0.05$ vs NC), 10.36 and 9.57 mg/mm, respectively, in the normal control, the colitis control, the positive control, and the test groups treated with the extract at 100, 200, and 400 mg/kg of body weight (Figure 3B).

The mean (SD) relative colon weights were 0.75 (0.02), 0.93 (0.07) ($P < 0.05$ vs NC), 0.88 (0.09) ($P < 0.05$ vs NC), 0.98 (0.04) ($P < 0.05$ vs NC), 1.23 (0.18) ($P < 0.01$ vs NC), and 0.92 (0.06) ($P < 0.05$ vs NC), respectively, in NC, CC, Lop5, and the test groups treated with the extract at 100, 200, and 400 mg/kg of body weight (Figure 3C).

*Effect of Bixa orellana leaves extract on hematological, leukocyte, and platelet parameters*

The hematological parameters, red blood cells (RBCs), hemoglobin (Hb), and hematocrit (Ht) for the normal control group were found to be 4.05 (0.19) $\times 10^6$/µL, 13.15 (0.66) g/dL, and 38.65% (1.92%), respectively. In the CC group, a nonsignificant decrease ($P > 0.05$) in these values (3.93 [0.14] $\times 10^6$/µL, 13.02

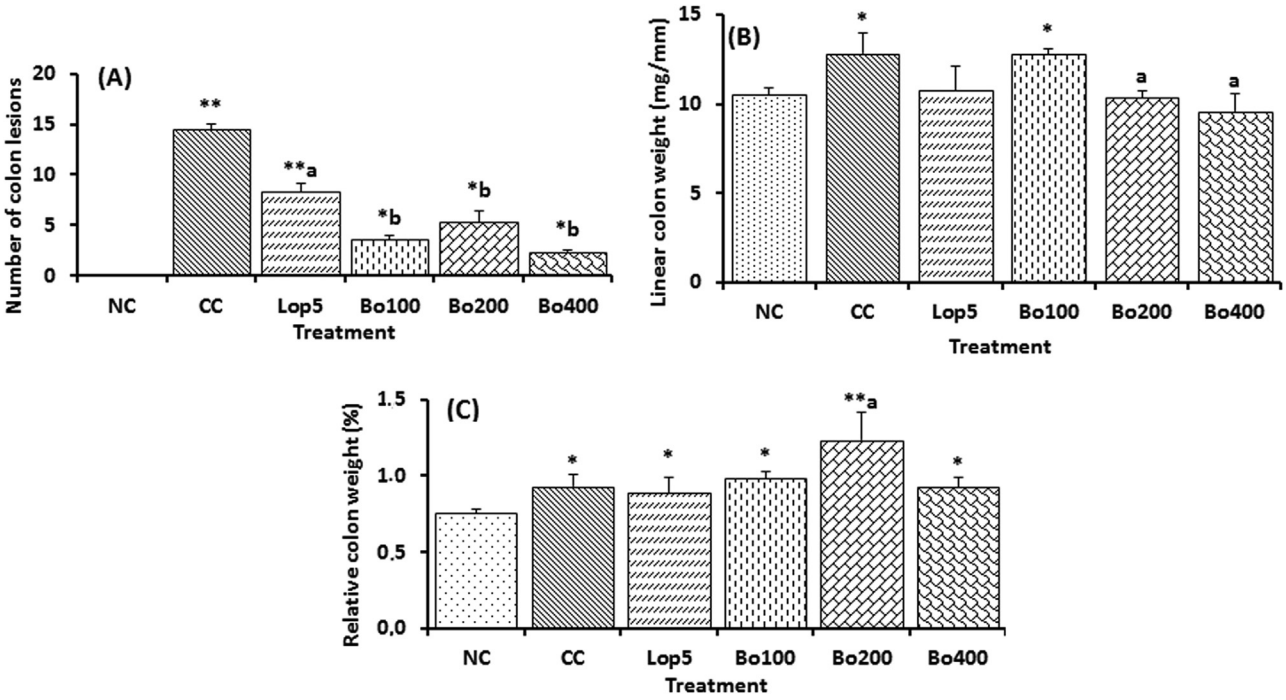


**Figure 3.** Number of colon lesions (A), linear weight (B), and relative weight (C) of colon in normal rats (NC), colitis control (CC), and rats treated with loperamide (Lop5) and hydroethanolic extract of *Bixa orellana* at 100 (Bo100), 200 (Bo200), and 400 (Bo400) mg/kg body weight. N = 6. Significant difference: $^{*}P < 0.05$; $^{**}P < 0.01$ between NC and other groups; $^{a}P < 0.05$; $^{b}P < 0.01$ between CC and treated groups.

**Table 1**
Hematological, leukocyte, and platelet parameters in normal rats (NC), the colitis control (CC) group, and rats treated with loperamide (Lop5) and with hydroethanolic extract of *Bixa orellana* at 100 (Bo100), 200 (Bo200), and 400 (Bo400) mg/kg body weight.

| Parameter* | NC | CC | Lop5 | Bo100 | Bo200 | Bo400 |
|---|---|---|---|---|---|---|
| RBC, $\times 10^6$/mm$^3$ | 4.05 (0.19) | 3.93 (0.14) | 4.42 (0.05) | 4.77 (0.14)†,‖ | 4.07 (0.10) | 4.67 (0.13)†,‖ |
| Hemoglobin, g/dL | 13.15 (0.66) | 13.02 (0.49) | 14.18 (0.17) | 15.47 (0.39†,‖ | 12.94 (0.39) | 14.24 (0.19)†,§ |
| Hematocrit, % | 38.65 (1.92) | 38.35 (1.37) | 42.45 (0.54) | 46.20 (1.63)‡,‖ | 38.96 (1.13) | 42.96 (2.13)†,§ |
| MCV, µm$^3$ | 95.37 (0.82) | 97.52 (0.92) | 96.01 (0.65) | 96.84 (1.15) | 95.75 (0.81) | 94.65 (0.71) |
| MCHC, g/dL | 34.02 (0.49) | 33.94 (0.52) | 33.41 (0.21) | 33.55 (0.58) | 33.21 (0.11) | 33.61 (0.21) |
| MCH, pg | 32.45 (0.53) | 33.09 (0.57) | 32.08 (0.16) | 32.47 (0.24) | 31.79 (0.35) | 32.59 (0.45) |
| WBC, $\times 10^3$/mm$^3$ | 4.48 (0.18) | 3.55 (0.21)† | 4.65 (0.08)[a] | 4.28 (0.37)§ | 4.32 (0.14)§ | 4.42 (0.12)§ |
| Lymphocyte, % | 67.83 (3.27) | 75.16 (3.30) | 40.16 (1.81)‡,‖ | 68.66 (1.96)§ | 63.40 (2.09)§ | 67.40 (2.09)§ |
| Monocytes, % | 0.16 (0.16) | 0.16 (0.16) | 0.50 (0.34) | 0.16 (0.16) | 0.60 (0.36) | 0.30 (0.16) |
| Neutrophils, % | 29.33 (3.30) | 23.00 (2.78)‡ | 56.83 (1.90)‡,‖ | 29.50 (2.14) | 33.60 (2.33)§ | 30.60 (2.33)§ |
| Eosinophils, % | 2.33 (0.42) | 1.16 (0.40)† | 2.00 (0.45) | 1.00 (0.36)† | 1.80 (0.34) | 1.84 (0.34) |
| Basophils, % | 0.33 (0.21) | 0.50 (0.22) | 0.50 (0.22) | 0.67 (0.21) | 0.60 (0.22) | 0.51 (0.22) |
| Platelets $\times 10^6$/mm$^3$ | 43.62 (0.69) | 38.72 (1.61)† | 46.34 (0.58)‖ | 40.46 (1.50) | 42.71 (1.17) | 41.72 (1.12) |

MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cells; WBC = white blood cells.

* Values are mean (SEM). N = 6.
† Significant difference at $P < 0.05$ between NC and other groups.
‡ Significant difference at $P < 0.01$ between NC and other groups.
§ Significant difference at $P < 0.05$ between CC and treated groups.
‖ Significant difference at $P < 0.01$ between CC and treated groups.

[0.49] g/dL, and 38.35% [1.37%]) was observed. In animals treated with Lop5 or with the extract at doses of 100 and 400 mg/kg, a significant increase ($P \leq 0.05$) in RBCs, Hb, and Ht compared with NC and CC was observed. However, the extract at 200 mg/kg showed no significant difference. Regarding the values of mean corpuscular volume, the mean corpuscular Hb, the mean corpuscular Hb concentration, no significant difference was observed between the different groups (Table 1).

Total white blood cells (WBCs), neutrophils, and eosinophils for the normal control group were found to be 4.48 [0.18] $\times 10^3$/µL, 29.33% [3.30%], and 2.33% [0.42%], respectively. These values were significantly ($P < 0.05$) decreased in the CC group. However, in the positive control group, the level of circulating neutrophils (56.83% [1.90%]) increased significantly ($P < 0.01$) compared with NC and CC groups. No significant difference ($P > 0.05$) was observed between the different groups in the monocytes and basophils levels. The number of lymphocytes was significantly increased ($P < 0.01$) in the CC group (75.16% [3.30%]) compared with the NC group (67.83% [3.27%]). In contrast, loperamide induced a significant decrease in lymphocytes (40.16% [1.81%]; $P < 0.01$) compared with NC and CC (Table 1).

The platelet count was 43.62 [0.69] $\times 10^6$/µL in the NC group and 38.72 [1.61] $\times 10^6$/µL ($P < 0.01$) in the negative control group. In animals treated with loperamide (46.34 [0.58] $\times 10^6$/µL;

$P < 0.01$) and with the extract at different doses, no significant increase in platelets was observed (Table 1).

*Effects of Bixa orellana extract on oxidative stress parameters and on NO production*

The SOD activity of the colitis control group was significantly ($P < 0.01$) decreased in serum (−21.12%), and in colon (−19.46%) compared with the normal control group. Blood SOD activity was 15.67 [0.21] ($P < 0.01$), 15.50 [0.34] ($P < 0.01$), 17.00 [0.25] ($P < 0.01$), and 17.50 [0.22] ($P < 0.01$) IU/L in rats treated with loperamide and hydroethanolic extract of *Bixa orellana* at 100, 200, and 400 mg/kg body weight, respectively. However, this SOD activity in the colon was 66.00 [0.89] ($P < 0.01$), 66.00 [0.89] ($P < 0.01$), 74.66 [1.68] ($P < 0.01$), and 78.00 [0.89] ($P < 0.01$) IU/g in rats treated with loperamide and hydroethanolic extract of *Bixa orellana* at 100, 200, and 400 mg/kg body weight, respectively (Table 2).

Catalase activity was significantly ($P < 0.01$) decreased in serum (−70.58%), and in the colon (−46.58%) in the colitis control compared with the normal control. The hydroethanolic extract of *Bixa orellana* at different doses significantly increased the catalase activity in the serum and in the colon, but with a more remarkable increase in the blood (42.62 [1.13]; $P < 0.01$) at 400 mg/kg body weight compared with the CC group (Table 2).

Serum GSH levels were 58.98 [1.08], 29.85 [0.94] ($P < 0.01$), 58.50 [1.73], 53.28 [0.80] ($P < 0.05$), 56.17 [1.49], and 56.93 [1.68] µmol/L, respectively, in NC, CC, and rats treated with loperamide and hydroethanolic extract of *Bixa orellana* at 100, 200, and 400 mg/kg body weight. However, these values in the colon were 114.17 [4.07], 63.63 [3.04] ($P < 0.01$), 107.59 [3.86], 71.71 [2.81] ($P < 0.01$), 116.42 [2.99], and 116.52 [3.42], respectively, in NC, CC, Lop5, Bo100, Bo200, and Bo400 mg/kg body weight (Table 2).

The concentration of MDA significantly increased ($P < 0.01$) in serum (+830.33%), and in colon (+154.91%) of CC rats compared to NC rats. The hydroethanolic extract of *Bixa Orellana* in different doses caused a significant decrease ($P < 0.05$) in the level of MDA in serum, and in the colon compared with the negative controls (Table 2).

In the blood, the NO level was 49.00 [0.54] µmol/L in NC rats and 144.54 [6.99] µmol/L ($P < 0.01$) in CC rats. In the treated animals, these levels were significantly reduced compared with CC rats and were 63.45 [1.59] ($P < 0.01$), 66.99 [2.91] ($P < 0.01$), 64.66 [2.66] ($P < 0.01$), and 60.35 [1.34] ($P < 0.01$) µmol/L, respectively, at Lop5, Bo100, Bo200, and Bo400. In colon, the NO level was 122.02 [1.33] µmol/g tissue in NC rats and 318.97 [7.11] ($P < 0.01$) in CC rats. In the treated animals, these levels were significantly reduced compared with the CC rats and were 197.39 [4.56] ($P < 0.01$), 181.12 [4.16] ($P < 0.01$), 127.52 [4.27] ($P < 0.01$), and 157.04 [3.06] ($P < 0.01$) µmol/g tissue, respectively, at Lop5, Bo100, Bo200, and Bo400 (Table 2).

*Effect of Bixa orellana leaves extract on the histopathology of the colon*

Microscopic observation of colon sections of the NC group showed intact epithelium without damage (Figure 4A). The inflamed colon in the colitis control group showed inflammatory granulomas (66.66%) in the submucosa (Figure 4B). In all other treated groups, the colon wall showed no damage.

## Discussion

We found in the model of experimental colitis induced by acetic acid, that the indices of inflammation were less pronounced in animals treated with different doses of *Bixa orellana* extract compared with the CC group. In previous studies, it was shown that the administration of *Bixa orellana* leaf powder at 750 mg/d in adult patients for 6 months did not cause severe adverse effects.[26,27] In this model of UC in rodents, the ROS, vasoactive amines, and eicosanoids are involved in the destruction of the colon structure and the mucous barrier by chemical stimulation, increased vessel permeability, increased inflammatory mediators, and promotion of fibrin hydrolysis.[16] Bloody and mucous-containing diarrhea, weight loss, relative and linear colon weight, and inflamed colon ulcerations are considered reliable and sensitive indicators of the severity of ulcerative colitis.[12,17] In the present study, treatment of acetic acid-induced colitis in rats with hydroethanolic extract of *Bixa orellana* leaves significantly reduced the frequency of diarrhea, relative and linear colon weight, number of gross lesions, and limited body weight loss compared with CC rats, indicating potential effectiveness against inflammatory colitis.

IBDs such as UC are associated with oxidative stress, and this stress has been identified as among the main contributors to its pathophysiology.[28] Acetic acid releases protons into the intracellular space, causing massive intracellular acidification resulting in immense epithelial damage that leads to acute inflammation. This acute inflammation usually results in a decrease in the activity of SOD, a decrease in the activity of catalase, a decrease in the level of reduced GSH, and an increase in the level of MDA, a marker of lipid peroxidation. All these parameters are associated with the release of proinflammatory mediators, resulting in an increase in NO

**Table 2**
Activities of superoxide dismutase (SOD) and catalase and levels of reduced glutathione (GSH), malonedialdehyde (MDA), and nitric oxide (NO) in serum and colon of normal control (NC), colitis control (CC), and rats treated with loperamide (Lop5) and hydroethanolic extract of *Bixa orellana* at 100 (Bo100), 200 (Bo200), and 400 (Bo400) mg/kg body weight.

| Tissues | Parameters* | NC | CC | Lop5 | Bo100 | Bo200 | Bo400 |
|---|---|---|---|---|---|---|---|
| Serum | SOD, UI/L | 17.33 (0.21) | 13.67 (0.21)† | 15.67±0.21)†,§ | 15.50 (0.34)†,§ | 17.00 (0.25)‖ | 17.50 (0.22)‖ |
| | Catalase, mmol/L | 52.62 (1.70) | 15.47 (1.07)‡ | 52.85 (2.47)‖ | 15.71 (0.52)‡ | 41.43 (2.05)†,‖ | 42.62 (1.13)†,‖ |
| | GSH, µmol/L | 58.98 (1.08) | 29.85 (0.94)‡ | 58.50 (1.73)‖ | 53.28 (0.80)†,‖ | 56.17 (1.49)‖ | 56.93 (1.68)‖ |
| | MDA, µmol/L | 1.22 (0.14) | 11.35 (0.64)‡ | 2.88 (0.09)‖ | 5.99 (0.05)†,§ | 2.06 (0.05)‖ | 1.58 (0.14)‖ |
| | NO, µmol/L | 49.00 (0.54) | 144.54 (6.99)‡ | 63.45 (1.59)†,‖ | 66.99 (2.91)†,‖ | 64.66 (2.66)†,‖ | 60.35 (1.34)†,‖ |
| Colon | SOD, UI/g tissue) | 75.33 (1.23) | 60.67 (1.23)‡ | 66.00 (0.89)‡,§ | 66.00 (0.89)‡,§ | 74.66 (1.68)‖ | 78.00 (0.89)‖ |
| | Catalase, mmol/g tissue | 347.62 (11.46) | 185.71 (6.39)‡ | 347.62 (11.46)‖ | 228.57 (7.37)†,§ | 233.33 (8.78)†,§ | 333.33 (15.93)‖ |
| | GSH, µmol/g tissue | 114.17 (4.07) | 63.63 (3.04)‡ | 107.59 (3.86)‖ | 71.71 (2.81)‡ | 116.42 (2.99)‖ | 116.52 (3.42)‖ |
| | MDA, µmol/g tissue | 16.28 (0.91) | 41.49 (1.53)‡ | 15.04 (0.96)‖ | 21.62 (1.23)§ | 12.35 (0.66)†,‖ | 14.57 (1.24)‖ |
| | NO, µmol/g tissue | 122.02 (1.33) | 318.97 (7.11)‡ | 197.39 (4.56)†,‖ | 181.12 (4.16)†,‖ | 127.52 (4.27)‖ | 157.04 (3.06)‖ |

* Values are presented as mean (SEM). N = 6.
† Significant difference at $P < 0.05$ between NC and other groups.
‡ Significant difference at $P < 0.01$ between NC and other groups.
§ Significant difference at $P < 0.05$ between CC and treated groups.
‖ Significant difference at $P < 0.01$ between CC and treated groups.

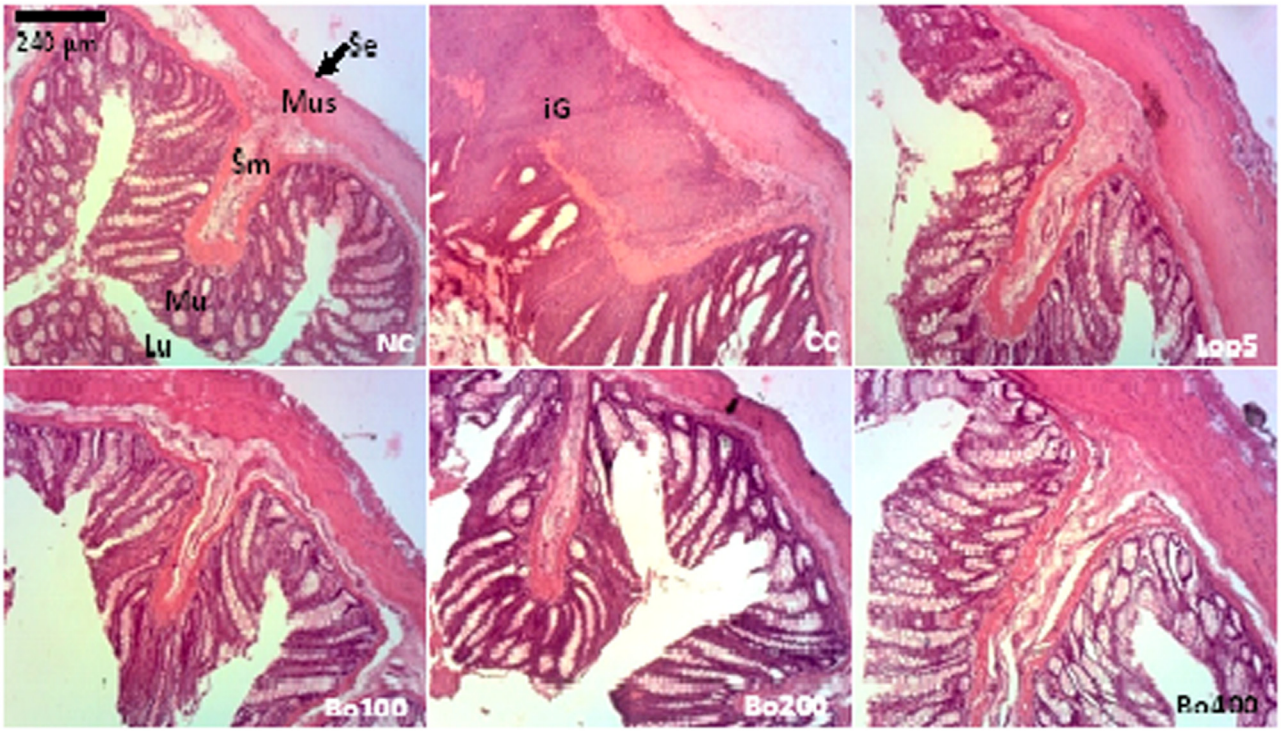


**Figure 4.** Photomicrograph of colon (hematoxylin and eosin stain ×40) of normal control, the colitis control, and rats treated with loperamide and hydroethanolic extract of *Bixa orellana* at 100, 200, and 400 mg/kg body weight. iG, inflammatory granuloma; Lu = intestinal lumen; Mu = mucosa; Mus = muscular; Se = serious; Sm = submucosa.

concentrations.[29] Acetic acid is an exogenous compound, that creates aggressive ROS responsible for oxidative stress.[30] Acetic acid is therefore considered to be a pro-oxidant, capable of increasing the activity of nicotinamide adenine dinucleotide phosphate oxidase responsible for the formation of free radicals in the mitochondria. These free radicals are at the origin of the lipid peroxidation that generates MDA, and of the drop in the level of GSH, due to the ability of GSH to neutralize free radicals through the action of glutathione peroxidase.[31] Rats with acetic acid-induced colitis treated with a hydroethanolic extract of *Bixa orellana* leaves had significantly increased SOD and catalase activities, GSH concentration and reduced MDA and nitrite concentrations. The leaves of *Bixa orellana* have been found to contain flavonoids (7-apigenin bisulfate, 7-luteolin bisulfate, and 8-bisulfate hypoletin); flavones; diterpenes (alkaloids and gallic acid); carotenoids (bixin, norbixin, orelin, beta carotene, lutein, cryptoxanthin, and zeaxanthin); and vitamins A, B, and C.[32] There are multiple ways these molecules can regulate oxidative stress by direct capture of ROS or by inhibition of certain enzymes responsible for the production of ROS such as cyclooxygenase and lipooxygenase.[33]

Among the most common extraintestinal complications and/or manifestations of UC has been reported to induce anemia due to iron deficiency.[34,35] Acetic acid-induced UC has induced anemia characterized by a decrease in Hb level accompanied by a reduction in RBCs and Ht level and a decrease in total WBCs and neutrophils. The minimal decrease in the level of RBCs, Hb, and Ht in the CC group compared with the NC group could be explained by the short duration of the study and/or by the influence of sex.[12] In normal women, estrogen plays an important role in the cyclical alteration of gastrointestinal symptoms during the menstrual cycle and during menstruation women with IBD tend to have more diarrhea.[36] However, the high values obtained in the treated groups could be explained by the presence of compounds with hematopoiesis activity because a previous study showed that the ethanolic extract of the leaves of *Bixa orellana* at 80 and 120 mg/kg body weight was reported to significantly increase the total number of RBCs and Ht levels in rats.[37] The extract at 200 mg/kg body weight showed no significant difference in the level of RBCs, Hb levels, and Ht levels, unlike the 100 and 400 mg/kg body weight, which increased these values. The reasons responsible for this discrepancy are not clear and should be explored in future studies. The extract via flavonoids[38] would inhibit intestinal inflammation, thus promoting iron absorption and stimulation of hematopoiesis.

It has been shown that during an inflammatory reaction, circulating polymorphonuclear cells (neutrophils, basophils, and eosinophils) infiltrate damaged tissues. This infiltration of neutrophils in the case of colitis, reduces the number of total circulating WBCs, as well as the number of circulating polynuclear cells.[39] The resulting decrease in the number of circulating polynuclear cells could explain the increased percentage of circulating lymphocytes that was observed.

## Conclusions

The hydroethanolic extract of *Bixa orellana* leaves had protective effects against acetic acid-induced colitis in rats that was associated with inhibition of the production of free radicals that are responsible for oxidative stress, hematological disorders, and tissue damage in this animal model.

## Conflicts of Interest

The authors have indicated that there is no conflict of interest regarding the content of this article.

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