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ORIGINAL ARTICLE

Effect of *Boswellia serrata* on Antioxidant Status in an Experimental Model of Colitis Rats Induced by Acetic Acid

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Abstract
Aim of the Study To evaluate the antioxidant effect of an extract of the plant *Boswellia serrata* in an experimental model of acute ulcerative colitis induced by administration of acetic acid (AA) in rats.
Materials and Methods The extract of *B. serrata* (34.2 mg/kg/day) was administered orally by gavage for 2 days before and after induction of colitis with AA diluted to 4 % and in a volume of 4 ml.
Results The anal sphincter pressure in the groups treated with *B. serrata* showed a significant increase compared to the colitis group ($P < 0.001$). Histological analysis of treated animals showed less edema with preservation of mucosal crypts. Lipid peroxidation showed a significant decrease in the treated groups compared to the colitis group ($P < 0.001$). The superoxide dismutase (SOD) enzyme activity showed a significant reduction in the treated groups compared to the colitis group ($P < 0.001$), the glutathione peroxidase (GPx) significantly increased in the treated groups compared to colitis group ($P < 0.05$), and the same was the result for enzyme activity glutathione (GSH; $P < 0.05$).
Conclusions The extract of *B. serrata* has active antioxidant substances that exert protective effects in acute experimental colitis.

Keywords Antioxidant · *Boswellia serrata* · Inflammation · Oxidative stress · Radical scavenger · Ulcerative colitis

Abbreviations

ANOVA	One-way analysis of variance
GPx	Glutathione peroxidase
GSH	Glutathione
IBD	Inflammatory bowel disease
iNOS	Inducible nitric oxide
LPO	Lipid peroxidation
NO	Nitric oxide
ROS	Reactive oxygen species
SOD	Superoxide dismutase
UC	Ulcerative colitis

Introduction

Inflammatory bowel disease (IBD) is characterized by chronic inflammation of the gastrointestinal tract, involving an unknown specific pathogen [1, 2]. Some etiological factors, including genetic, immunological, and environmental, have been implicated in the pathophysiology of the disease. Thus, included in this classification is ulcerative colitis (UC), which involves only the colon and rectum and is characterized by leukocyte infiltration in the mucosa and superficial ulcers [3].

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The leukocyte infiltration in UC is due to colonic barrier rupture and invasion of bacterial and antigenic stimuli, with a release of inflammatory mediators, such as cytokines and arachidonic acid metabolites, and release of oxygen free radicals, which can lead to oxidative damage [4, 5]. In the study of Tüzün [6], it was found that the reactive oxygen species (ROS) and nitrogen species are overproduced in patients with colitis causing adverse effects, such as lipid peroxidation of membrane and attack on tissue proteins and DNA [7].

The organism has defenses against ROS which are known as antioxidants. They are divided into two main types: enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), and non-enzimatic: glutathione (GSH), ascorbic acid (vitamin C), and flavonoids, among others. The function of these enzymes is to maintain the levels of ROS in low concentrations, thereby acting to prevent the formation of such species [8, 9].

Thus, it is necessary to find substances that can inhibit, prevent, or ameliorate the damage caused by reactive oxygen species produced in colitis; antioxidants with anti-inflammatory action are consistent with the decrease of damage caused by these substances [10].

Some plants have antioxidant properties for the organism, so they are indicated for the treatment and prevention of many diseases, such as the plant *Boswellia serrata* (*B. serrata*) from India. It is part of a group of angiosperms, the family Burseraceae, which contains phenolic compounds that are characterized by a benzene ring, a carboxyl terminus, and one or more hydroxyl groups and/or methoxyl in the molecule [11, 12]. It confers antioxidant properties on the organism. The extract of *B. serrata*, taken from its trunk, has been used in traditional medicine in India and other eastern countries to treat inflammatory diseases, such as arthritis, osteoarthritis, and IBD, and is also effective in preventing lipid oxidation [13].

The active substance in the extract of *B. serrata* is known as boswellic acid (BA), which is characterized by pentacyclic triterpenes containing antioxidant properties [12], such as anti-inflammatory, anti-atherosclerotic, anti-hepatotoxic, and anti-hyperlipidemic [14]. These active ingredients act as free radicals scavengers, and sometimes as metal chelators, acting in both as the initiation step in the propagation of the oxidative process [15].

As already demonstrated in several studies, an excessive production of ROS and inflammation occurs in UC, leading to oxidative damage in tissues, so it is necessary to seek antioxidant therapies to reduce the damage generated. In this study, we evaluated the antioxidant capacity of *B. serrata* to reduce oxidative and tissue damage in experimental colitis by acetic acid in rats.

Materials and Methods

Animals

The study used 25 male Wistar rats weighing 300 g. They were divided into five groups: control (CO), colitis (CL), control + Boswellia (CO + B), colitis + Boswellia (CL + B), and Boswellia + colitis (B + CL). The animals were kept in the vivarium of the Lutheran University of Brazil (ULBRA) during the experiment, in a cycle of 12 h light/dark and temperature between 20 and 25 °C. Water and food were given ad libitum. The model chosen for the induction of colitis was adapted from those described by Yamada [16] and Tannahill et al. [17]. The animals received intracolonic administration of acetic acid 4 % in a volume of 4 ml by enema. The groups received extract of *B. serrata* orally, 34.2 mg/kg/day, corresponding to the dose of *B. serrata* extract/kg applied in a previous study by Krieglstein et al. [18], 48 h before and after induction of colitis once a day until the end of the experiment. The drug used in this experiment was from the laboratory of the Vedic Apsen Fitomedicine where 350 mg of dry extract of *B. serrata* Roxb. ex Colebr–Burseraceae corresponds to 3 mg AKBA acid (3-acetyl-11-keto-β-boswellic acid). The active are isolated boswellic acids, among them the 3-acetyl-11-keto-β-boswellic acid (AKBA), the active principle, which is more relevant and potent in inhibiting the action of leukotrienes, through direct action on the 5-lipoxygenase. The pharmacokinetics of AKBA include a peak plasma level of 4.5 ± 0.55 h, the half-life is determined as $\neq 5.97$ 0.95 h, the mean volume of distribution is 22 ± 142.87, 78 l, and the clearance is 296.10 ± 0.9 ml/min.

The death of the animals was performed after pressure measurements. For the anesthesia of the animal, we used xylazine hydrochloride 50 mg/kg and ketamine hydrochloride 100 mg/kg body weight intraperitoneally for removal of the distal colon (8 cm). After that, euthanasia was performed by exsanguination under anesthesia.

Experiments followed a protocol observed by the Animal Ethics Committee of the Lutheran University of Brazil (ULBRA) with the recommendations of the European Union regarding animal experimentation: Directive of the European Counsel 86/609/EEC [19].

Anal Sphincter Pressure Measurements

Before the euthanasia, the animals were lightly anesthetized with Isoflurane® to perform the anal sphincter pressure measurement. We performed anorectal manometry (Proctossystem; Viotti, SP) with a balloon catheter and measured in cm of H_2O. Three measurements were subsequently made in each animal [20].

Histological Analysis

For histological examination, a portion of the intestine was placed in buffered formalin. Later, they were included in paraffin blocks, after being cut on a rotary microtome at a thickness of 3 μm. We performed staining with hematoxylin-eosin (HE) for histological usual. The slides were analyzed in LABOPHOT NIKON binocular microscope at a magnification of ×200.

Intestine Homogenates

The intestines were weighed and homogenized for 30 s in an Ultra-Turrax (IKA-WERK) for 40 s at 4 °C in the presence of 1.15 % KCl (9 ml per g of tissue) and methyl phenyl sulfonyl fluoride (PMSF) at a concentration of 100 mM in isopropanol (10 μl per ml of KCl added). Then, the homogenates were centrifuged for 10 min at 3,000 rpm in a refrigerated centrifuge (SORVALL Super T21; Condensed Operating Kendro Laboratory Products, USA). The supernatant was pipetted into Eppendorf flasks, and the precipitate was discarded. The samples were stored again at −80 °C for posterior analyses [21].

Protein

The proteins were quantified by the method described by Lowry and colleagues, using as a standard solution bovine albumin at a concentration of 1 mg/ml. The samples were measured spectrophotometrically at 625 nm, and values expressed in mg/ml. The values were used to calculate values of TBARS (thiobarbituric acid–reactive substances) and antioxidant enzymes [22].

Lipid Peroxidation

The amount of aldehydes generated by lipid peroxidation was measured by the TBARS method, which measures the amount of substances reacting with thiobarbituric acid. The samples were incubated at 100 °C for 30 min after addition of 500 μl of 0.37 % thiobarbituric acid in 15 % trichloroacetic acid, and centrifuged at 3,000 rpm (1,612.8*g*) for 10 min at 4 °C. Absorbance was determined spectrophotometrically at 535 nm [23].

Antioxidants Enzyme Analyses

The analysis of superoxide dismutase (SOD) is based on the inhibition of the reaction of the superoxide radical with adrenaline, detected spectrophotometrically at 480 nm, and values expressed in U/mg prot [24]. The activity of glutathione peroxidase (GPx) is based on the consumption of NADPH in the reduction of oxidized glutathione, detected spectrophotometrically at 340 nm for 3 min, and values expressed in mmoles/min/mg prot [25]. The measurement of glutathione (GSH) was detected spectrophotometrically at 412 nm, and values expressed in μmol/mg prot., made according to the method of Beutler et al. [26].

Statistic Analysis

All data are presented as means ± SE. Statistical significance was calculated using Graphpad Instat, v.3.0 for Windows. We used variance analysis (ANOVA) and Student's–Newman–Keuls test for multiple analysis, adopting a significant level of 5 % ($P < 0.05$).

Results

Histology

The slides were stained with hematoxylin-eosin (HE) and analyzed at ×200 magnification. Figure 1a shows a photomicrograph of an animal in the control group (CO) in which we can observe the integrity of the crypts (CP) with simple glandular epithelium and normal submucosa (SM). Figure 1b is a photomicrograph of an animal of control *Boswellia* group (CO + B) showing similar architecture to the control group. Figure 1c is a photomicrograph of an animal of the colitis group (CL) showing changes in the architecture of the colon, destruction of CP, submucosal edema (E), and inflammatory infiltrate (IF). Figure 1d is a photomicrograph of an animal of the prophylactic treatment group (B + CL) in which we can observe a preservation of CP and inflammatory infiltrate. Figure 1e is a photomicrograph of an animal of the colitis group treated with *B. serrata* (CL + B) in which we can observe a preservation of CP with glandular epithelium, and less inflammatory infiltrate.

Anal Sphincter Pressure and Lipid Peroxidation

The results of the anal sphincter pressure showed a significant increase in the treated groups (CL + B and B + CL) compared to the colitis group ($P < 0.001$; Fig. 2a). The analysis of the values of lipid peroxidation (LPO) obtained by TBARS showed a significantly decreased of LPO in the treated groups (CL + B and B + CL) compared to the colitis group ($P < 0.001$; Fig. 2b).

Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx) and Glutathione (GSH)

The SOD activity decreased significantly in the treated groups (CL + B and B + CL) compared to the colitis group ($P < 0.001$; Fig. 3a). The GPx (Fig. 3b) and GSH activity (Fig. 3c) increased ($P < 0.05$) in treated groups (CL + B and B + CL) compared to colitis group.

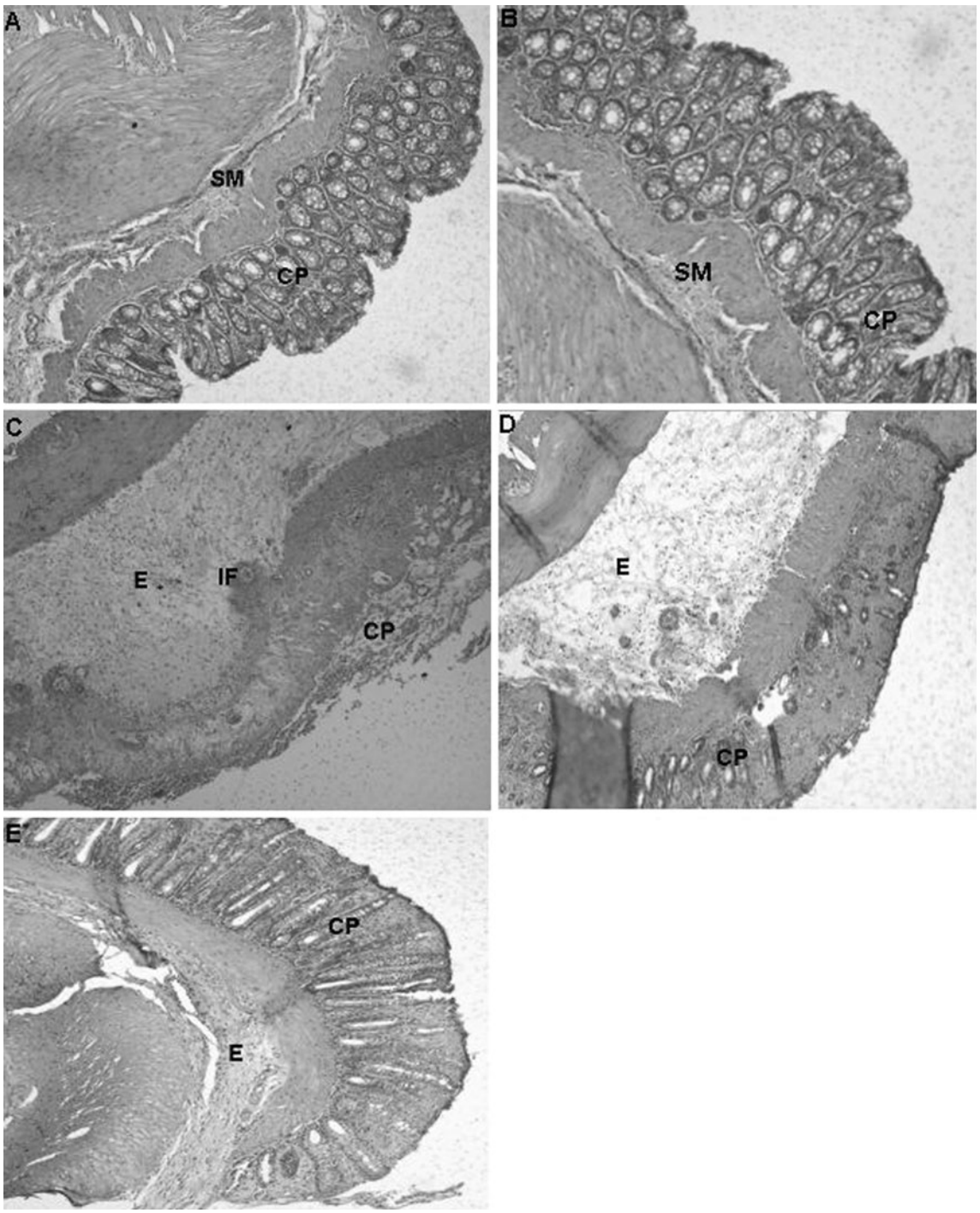

Fig. 1 Effect of administration of *B. serrata* on colon injury in colitis model in rats induced by acetic acid. **a** Control group (CO), **b** control *Boswellia* group (CO + B), **c** colitis group (CL), **d** prophylactic treatment with *B. serrata* (B + CL), **e** colitis group treated with *B. serrata* (CL + B). Crypts (*CP*), submucosa (*SM*), edema (*E*), inflammatory infiltrate (*IF*)

Discussion

The etiology of the ulcerative colitis has not yet been well determined. For this reason, several experimental models of colitis are in use, using a model with features of toxic and acute presentation. The acetic acid causes an injury in the intestines of animals, developing an inflammation that is considered one of the most prominent features found in colitis [27]. The extract of *B. serrata* has active ingredients that contain antioxidants with anti-inflammatory action and are distinguished by preventing the synthesis of proinflammatory cytokines such as leukotrienes. They inhibit the

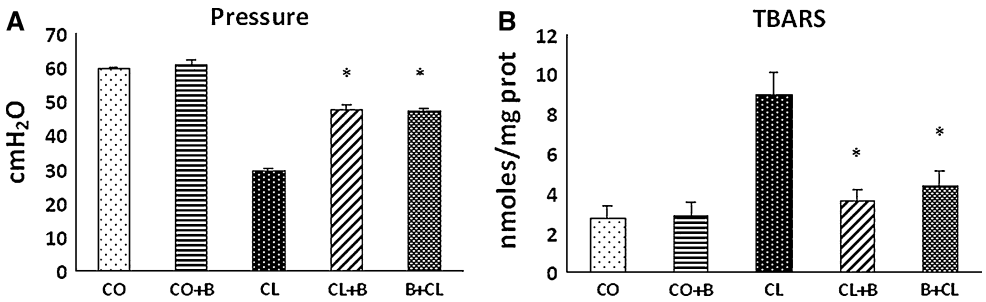

Fig. 2 Effect of administration of *B. serrata* on anal sphincter pressure and lipoperoxidation in colitis model in rats induced by acetic acid. Data are expressed as the means ± SEM. **a** *Pressure* (cmH_2O), **b** lipoperoxidation (*TBARS* nmoles/mg prot). *Asterisk* significant difference between groups CL + B and B + CL and the CL group ($P < 0.001$). *CO* control group, *CO + B* control group + *B. serrata*, *CL* colitis group, *CL + B* colitis + *B. serrata*; *B + CL B. serrata* + colitis

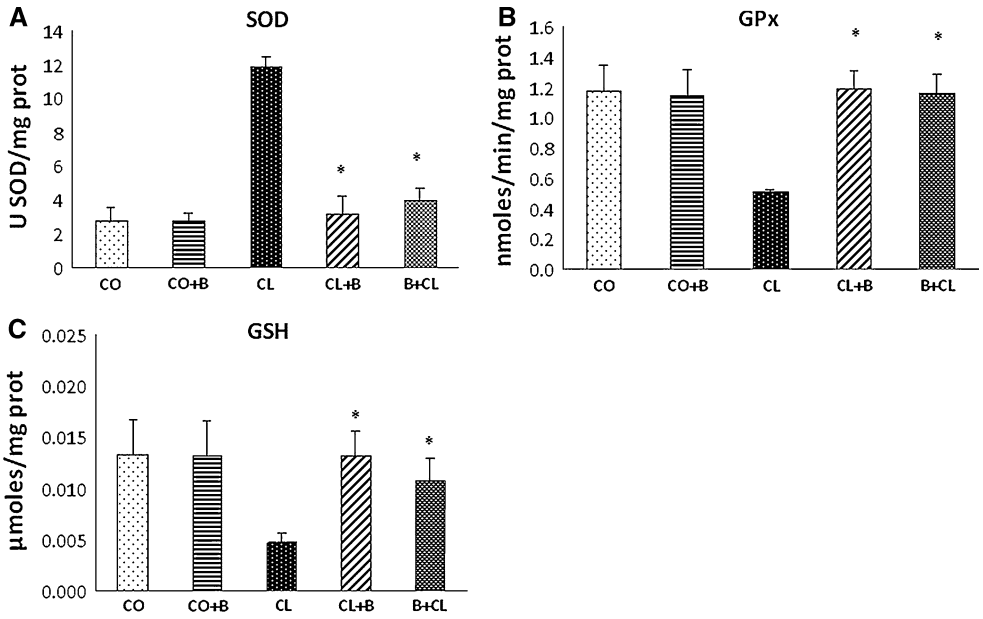

Fig. 3 Effect of administration of *B. serrata* on the activity antioxidants enzyme SOD (U sod/mg prot) (**a**), GPx (nmoles/min/mg prot) (**b**), and GSH (µmoles/mg prot) (**c**) in a colitis model in rats induced by acetic acid. Data are expressed as the means ± SEM. *Asterisk* significant difference between groups CL + B and B + CL and the CL group. SOD ($P < 0.001$), GPx and GSH ($P < 0.05$). *CO* control group, *CO + B* control group + *B. serrata*, *CL* colitis group, *CL + B* colitis + *B. serrata*; *B + CL B. serrata* + colitis

enzyme 5-lipoxygenase [15, 18, 28]. The study by Anthoni et al. [29] indicated that boswellic acid 3-acetyl-11-keto-beta-bosvélico (AKBA) significantly attenuated leukocyte recruitment, thus protecting the intestinal mucosa against tissue injury caused by indomethacin, which was used to induce the experimental of colitis.

Histological evaluation of tissue from the intestines confirmed the development of inflammation in the colitis group. We observed a destruction of mucosal crypts, and significant edema in the submucosa with inflammatory infiltrate; similar results were found by Fillmann et al. [30]. The animals treated with *B. serrata* showed a partial preservation of the crypts and less mucosal edema. In animals previously treated with *B. serrata*, we also observed an attenuation of this phenomenon. These findings are consistent with other studies using antioxidants such as glutamine, superoxide dismutase, *Abarema cochliacarpos, B. serrata*, and *Scutellaria baicalensis* [31–34]. The organism reaction to aggressive agents used in experimental models of colitis such as acetic acid (AA), 2,4,6 trinitrobenzene sulfonic acid (TNBS), indomethacin, and dextran sodium sulfate (DSS) [27, 35] promote vasodilation and leukocyte chemotaxis, as well as an increase in blood flow, which generates a high production of oxygen, hence the excessive production of reactive oxygen species (ROS) and nitrogen [36]. However, the colitis is an imbalance between oxidant and antioxidant substances characterizing oxidative stress [37]. The ROS induce redox process of LPO, which is a chain reaction that acts primarily on the lipid membrane that spread in phases of initiation, propagation, and termination [8]. In this study, we measured the LPO, and found a significant decrease in the treated groups when compared with the colitis group. Therefore, we suggest that the plant extract *B. serrata* was effective in reducing the production of reactive oxygen species, possibly due to its antioxidant activity.

There are some studies using treatments with antioxidants with the reduction of LPO, as in the study of

Kretzmann et al. [30], where we observed a decrease of LPO in the group treated with glutamine compared to the colitis group, and the study by Lee et al. [38] who used the compound Berberine extract which has antioxidant properties with anti-inflammatory action. The results of treatment with Berberine showed an inhibition of lipid peroxidation in animals of this group, suggesting the antioxidant effect of the plant *Mahonia aquifolium* containing the compound studied.

The internal anal sphincter is a smooth muscle that relaxes under the inhibitory control of nitric oxide (NO). The release of iNOS in ulcerative colitis and the consequent increase of NO in response to a stimulation of neurons and non-adrenergic non-cholinergic (NANC) show a relaxation of the sphincter muscles [39], causing a decrease in anal sphincter pressure levels. The colitis group showed a significant decrease in anal pressure when compared to the other groups. However, the groups CL + B and B + CL showed a significant increase in anal sphincter pressure when compared to the colitis group. This suggests that animals with colitis had an anal sphincter muscle relaxation; similar results were found in the study of Fillmann et al. [40, 41] with experimental models of colitis and diabetes in rats [42].

The antioxidant system is a compensatory mechanism for the oxidation process consisting of enzymatic and non-enzymatic substances working against oxidative damage. The enzyme system, such as SOD and GPx, prevents the accumulation of superoxide anion ($O_2^{\cdot}$) and hydrogen peroxide (H_2O_2), and is therefore considered the primary line of defense. These enzymes are not found in the cytosol but in mitochondria, where most free radicals are produced. However, the increase in SOD may result in increased formation of H_2O_2, which may cause an accumulation of H_2O_2 in the tissues, inducing oxidative stress [43].

In our study, we observed a significant increase in SOD activity in the colitis group, probably to compensate for the damage caused by the action of acetic acid in the animals' intestine. The enzyme SOD has an essential role in cellular redox balance, promoting dismutate in an attempt to free radicals, protecting tissues against oxidative damage [44]. In contrast, animals with colitis who were treated with *B. serrata* kept the values of SOD enzyme activity close to the control group. In the study by John et al. [45], the oxidative damage in erythrocytes of animals caused by dimethoate also showed an increase in SOD activity in the group exposed to the insecticide and as in our study.

Glutathione peroxidase (GPx) has a great physiological importance because it catalyzes the decomposition of inorganic peroxide and organic peroxides, using GSH as co-substrate. In our study, we observed a significant increase in groups CL + B and B + CL group compared to CL. Studies suggest that GPx is responsible for the detoxification of (H_2O_2), when it is present at low concentration [46]. The reduction of GPx activity in the intestine of animals with colitis is blocked after administration of antioxidant [47]. This was also verified in this study. Glutathione (GSH) is a key component protecting against damaging of free radicals in the physiological system [48]. In our results, the enzyme GSH showed a significant increase in the treated groups CL + B and B + CL. A similar result was demonstrated in the study by Tahan et al. [49], which administered doses of melatonin in the colitis group and also observed an increase in the enzyme GSH.

In conclusion, the study results suggest that *B. serrata* acts by inhibiting lipid peroxidation, and its antioxidant effect is significant in the restoration of the enzymes. However, it also showed a significative improvement in the colon inflammation of animals with colitis induced by acetic acid. Thus, the results justify its use for the treatment of gastrointestinal diseases, but more detailed studies should be conducted in order to evaluate the protective effect in humans.

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Conflict of interest None.

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