Effects of *Boswellia serrata* Roxb. and *Curcuma longa* L. in an in vitro intestinal inflammation model using immune cells and Caco-2

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**Abstract:** Inflammatory bowel diseases, which consist of chronic inflammatory conditions of the colon and the small intestine, are considered a global disease of our modern society. Recently, the use of herbal therapies has increased in patients with inflammatory bowel diseases because of their effectiveness and better safety profile, compared to conventional drugs. *Boswellia serrata* Roxb. and *Curcuma longa* L. are amongst the most promising herbal drugs, however, their clinical use in inflammatory bowel diseases is limited and little is known on their mechanism of action. The aim of this work was to investigate the effects of two standardized extract of *B. serrata* and *C. longa* in an in vitro model of intestinal inflammation. Their impact on cytokine release and reactive oxygen species production, as well as the maintenance of the intestinal barrier function and on intestinal mucosa immune cells infiltration, has been evaluated. The extracts showed a good protective effect on the intestinal epithelium at 1 µg/ml, with *C. longa* having an anti-inflammatory mechanism of action and *B. serrata* acting as an antioxidant. In summary, these herbal products were demonstrated to be promising agents for the management of inflammatory bowel diseases by modulating in vitro parameters which have been identified in the clinical conditions.

**Keywords:** *Boswellia serrata* Roxb.; *Curcuma longa* L.; intestinal bowel diseases (IBD); Caco-2; PBMC; HMC-1.1; mast cells; cytokines; trans epithelial electrical resistance (TEER); reactive oxygen species (ROS)

1. Introduction

Inflammatory bowel diseases (IBDs) are a group of diseases very common in modern society, correlated with strong inflammatory conditions of the colon and small intestine [1]. The actual aetiology of IBDs is still unknown [2]. The incidence of IBDs in the 20th century was thought to be limited to Western countries [3], however, in the 21st century the incidence of IBDs has increased throughout the world, making it an important global disease [4].

IBDs are characterized by aberrant immune responses against microorganisms which are present in the intestine. Mast cells may play a critical role in IBDs pathogenesis, since they are typically located just beneath the intestinal mucosal barrier and can be activated by bacterial
antigens. These cells can potentially contribute to IBDs through their effects on immunoregulation [5].

The current pharmacological treatments for IBDs focus on the use of drugs, such as 5-aminosalicylates, corticosteroids, immunosuppressive and biological agents, able to reduce inflammation and related symptoms [6]. However, these drugs present some side effects such as rash, nausea and vomiting, which limit their therapeutic application [7].

Herbal therapies have been used since ancient time to treat a wide variety of diseases [8,9] and can also represent a valid alternative to conventional treatments in IBDs due to their validated effectiveness and better profile of safety.

Among the multiple phytotherapeutic agents available, Curcuma longa L. rhizome (turmeric) and Boswellia serrata Roxb. gum resin (boswellia) are considered among the most promising herbal drugs for the management of IBDs [10].

According to the European Medicines Agency, C. longa root has a therapeutic indication derived from traditional use for the relief of gastrointestinal disorders [11]. Curcuminoids are able to inhibit lipoxygenases, cyclooxygenases and phospholipases and act on AP-1, STAT and NF-κB pathways [12,13]. Furthermore, an important free radical scavenging activity has been associated with the biological activity of turmeric [14].

In 2014, McCann and colleagues showed that curcumin-enriched turmeric extracts were able to increase the activity of an IL-10 promoter variant associated with IBD in human embryonic kidney cells [15]. Moreover, curcumin inhibited the proliferation of splenocytes as well as IL-4 and IL-5 secretion by CD4(+1) lymphocytes in a mouse model of chemically-induced colitis [16]. The same group, then, demonstrated that curcumin can attenuate the release of MIP-1α, MIP-2 and IL-1β from colonic epithelial cells and macrophages, as well as the release of IL-8 from neutrophils [17].

According to the World Health Organization, boswellia use for the management of IBDs is supported by clinical use [18]. The anti-inflammatory activity of boswellia is related to the inhibition of lipoxygenases and NF-κB [19]. Furthermore, the inhibition of lipid peroxidation and the increase of superoxide dismutase levels, which contribute to the antioxidant activity of boswellia, were correlated to the intestinal anti-inflammatory effect observed in an in vivo colitis model in rats [20,21].

Despite their biological potential, the clinical use of turmeric and boswellia in IBDs is still limited and controversial because of the lack of registered drugs in many countries and the variability of preparations used in clinical trials.

These reasons led us to investigate the in vitro effectiveness and the protective mechanisms of action of two dry extracts of Curcuma longa L. rhizome (CUR), standardized in curcumin, and Boswellia serrata Roxb. gum resin (BOS), standardized in boswellic acids, in maintaining the intestinal barrier integrity, using an innovative protocol that considered their action in human epithelial colorectal cells as well as immune cells (i.e. peripheral blood mononuclear cells and mast cells).

2. Results

2.1. Chemical analyses of dry extracts

Curcumin and boswellic acids content of dry extracts accomplished the values declared in products labels.

Curcumin content in CUR, according to Ph. Eur. 9th method, resulted 56.85% ± 2.79%, whereas total triterpenes content in BOS was 68.41% ± 3.33%.

2.2. Inflammatory model on Caco-2, PBMC and HMC-1.1: cytokines dosages

Firstly, we verified that, as reported in literature, intestinal inflammation is not related to cytokine release by intestinal epithelial cells [22]. We used Caco-2 cells as a model of the intestinal lumenal epithelium.

Measurement of cytokines released by Caco-2, after stimulation with a pro-inflammatory concentration of bacterial lipopolysaccharide (LPS) revealed IL-6, IL-8 and IL-10 levels were not
modified by LPS stimulation (500 ng/ml) but TNF-α was upregulated 1.38 fold compared to untreated controls (figure 1).

![Figure 1](image)

**Figure 1.** Cytokine release in Caco-2 cells; *p<0.05 vs control (set at 1).

Despite the poor release of cytokines by epithelial cells *in vitro*, cytokine levels are considered pivotal in maintaining and promoting IBDs such as ulcerative colitis and Crohn’s disease. The high levels of cytokine production observed during intestinal inflammation are probably due to infiltrating immune cells [23].

For this reason we focused our attention on a LPS-induced inflammation model using peripheral blood mononuclear cells (PBMC) and we investigated the anti-inflammatory activity of CUR and BOS.

The inflammatory model using PBMC confirmed a significant impact on production of cytokines following LPS stimulation. Notably TNF-α was over than 50 fold up-regulated compared to the control in each experiment we performed. IL-6 also was strongly up-regulated (over 15 folds compared to the control) (figure S2)

IL-8 up-regulation induced by LPS less striking compared to other cytokines but was statistically significant and confirmed that this chemo-attractant was induced by LPS (figure). Neither CUR nor BOS at any of the tested concentrations modulated LPS-stimulated TNF-α and IL-6 production by PBMC (figure S1).

In contrast, IL-8 up-regulation induced by LPS was inhibited by both CUR and BOS (figure 2); with significant reductions seen with CUR at 1 and 10 µg/ml which inhibited IL-8 release by 14.84% and 13.23%, respectively, compared to LPS-stimulated cells. At the lowest concentration used (100 ng/ml), CUR was not effective in inhibiting IL-8 release in stimulated PBMC.

Likewise, BOS inhibited IL-8 release in stimulated PBMC at each of the tested concentrations, even if statistically significance was obtained only at 1 µg/ml and at 10 µg/ml, with a reduction of at 9.32% and 11.90%, respectively, compared to LPS groups (figure 2).
Additionally the release of IL-10, the most representative regulatory cytokine, was monitored in non-inflammatory conditions. IL-10 release by PBMC incubated with CUR was up-regulated by 19.52% compared to the control group. On the contrary, BOS had no effects on IL-10 (figure 3).

As a result of the preceding experiments with PBMC, the concentration of 1 µg/ml, which was the minimum effective concentration, was chosen for further experiments.

TNF-α release modulation after LPS stimulation and CUR and BOS treatment on Caco-2 cells was re-evaluated (figure 4). It was noticeable that incubation with either CUR or BOS was effective in inhibiting LPS-induced TNF-α in Caco-2 by 25.60% and 13.82%, respectively.

Figure 2. IL-8 release in PBMC; *** p<0.001 vs control; # p<0.05 vs stimulus.

Figure 3. IL-10 release in PBMC; * p<0.05 vs control.
Afterwards, in order to better understand the modulation of cytokines observed in PBMC, which are composed of several immune cell subpopulations, we analysed the behaviour of a specific immune cell line involved in IBDs. Hence, we evaluated the release of inflammatory cytokines such as IL-6, IL-8, IL-10 and TNF-α by mast cells (HMC-1.1), as these are believed to play a crucial role in inflammatory conditions.

Stimulation of HMC-1.1 cells with phorbol 12-myristate,13-acetate (PMA) resulted in increased secretion of TNF-α, IL-6, and IL-8 by 4, 8 and 4.5 folds, respectively, compared to the untreated control. Interestingly, IL-6, the most abundant PMA-induced cytokine produced by HMC 1.1 was significantly inhibited by both CUR and BOS, by 39.44% and 39.06%, respectively (figure 5).

Neither CUR nor BOS at each of the tested concentrations modulated the release of TNF-α and IL-8 in HMC-1.1 stimulated by PMA (not shown).

IL-10 release was monitored too, but no variation was detected after the treatment of HMC-1.1 cells with CUR and BOS (not shown).
2.3. Measurement of ROS production

Since reactive oxygen species (ROS) production is common in every cell line and it is a typical phenomenon involved in inflammatory conditions [24], we analysed the modulation of ROS by CUR and BOS in Caco-2 cells.

Our analysis showed that H$_2$O$_2$ increased the ROS production in Caco-2 by 3.83 fold compared to the control. BOS 1 µg/ml decreased the ROS production by 24.33% compared to H$_2$O$_2$, while CUR 1 µg/ml was ineffective in inhibiting ROS production after H$_2$O$_2$ stimulation (figure 6).

![Figure 6. ROS release in Caco-2 cells; *** p<0.001 vs control; # p<0.05 vs stimulus.]

2.4. Intestinal permeability: TEER measurements

As described before, high levels of cytokines and leukocyte activation could be better framed as a consequence of an alteration in intestinal mucosa integrity and, thus, of increased epithelial permeability. Hence, we evaluated the integrity of the intestinal epithelium barrier by measuring the trans-epithelial electrical resistance (TEER) in LPS-stimulated Caco-2 cells, in order to assess the protective effect of BOS and CUR (figure 7).

![Figure 7. TEER measurement in Caco-2 cells. *** p<0.001 stimulus vs control; oooo p<0.001 CUR vs stimulus; ### p<0.001 BOS vs stimulus; # p<0.05 CUR vs stimulus.]

TEER is expressed as the percentage of resistance, normalized to the initial value.
The treatment with LPS decreased the TEER value by 55.04% and 65.51% compared to the not treated control, after 21 and 24 hours, respectively.

CUR almost completely protected the intestinal barrier, increasing the TEER value by 43.73% after 21 hours and by 32.98% after 24 hours, compared to LPS.

The pretreatment with BOS enhanced the TEER value by 34.33% and 19.41%, after 21 and 24 hours, respectively, compared to LPS, thus, significantly reverting the epithelial damage caused by the inflammatory stimulus.

2.5. Leukocytes infiltration: PBMC adhesion assay

Immune cell infiltration is a critical event for the establishment of intestinal inflammation. By developing a co-culture between LPS-stimulated Caco-2 and PBMC, we tried to simulate intestinal inflammation, evaluating the effect of CUR and BOS pretreatment.

LPS stimulation resulted in a readily detectable leukocyte infiltration, represented by the blue stained spots (figure 8b), that was inhibited by the treatment with CUR or BOS (figure 8c and 8d).

In agreement with the TEER measurements, the adhesion assay demonstrated a strong protective effect of CUR and BOS on intestinal epithelium.

![Figure 8](#)

**Figure 8.** PBMC adhesion assay: (a) control, (b) LPS 500 ng/ml, (c) CUR 1 µg/ml + LPS 500 ng/ml, (d) BOS 1 µg/ml + LPS 500 ng/ml.

3. Discussion

IBDs are strictly correlated with intestinal barrier dysfunctions, which lead to a strong antigenic response, characterized by oxidative damage and inflammation [25].

In this study CUR and BOS both demonstrated a strong protective effect on the epithelial barrier by increasing TEER values and reducing immune cells infiltration. These data are in
agreement with our previous experiments for boswellia [26] but also suggest a novel interesting biological effect of turmeric.

The co-culture of Caco-2 cells and PBMC resulted in a dynamic and more realistic picture of bowel inflammatory conditions, by showing how blood mononuclear cells participate in the infiltration process linked to modified integrity of inflamed intestinal epithelial cells.

In our model, apart from a slight increase in TNF-α levels, we could not detect a change in cytokine levels in LPS-stimulated Caco-2 compared with the non-treated control. Indeed, intestinal inflammation appears not to be related to cytokine release by epithelial cells. In Caco-2, this is possibly due to the low level of TLR-4 receptors expressed. These receptors, in fact, are mainly expressed at the crypts rather than at the lumen, and mediate the response to the LPS stimulus, resulting in NF-κB signal activation [22]. On the contrary, immune cells are largely responsible for intestinal cytokines accumulation in IBDs [27]. For these reasons, we used immune cells in order to simulate an inflammatory environment, with the aim of better understanding the anti-inflammatory mechanism(s) of action of CUR and BOS in IBDs.

The comprehensive analysis of cytokines modulated by CUR and BOS after inflammatory stimuli on the different cell types revealed that the tested samples had some peculiar anti-inflammatory effectiveness at the concentration of 1 µg/ml. In particular, both CUR and BOS were effective in reducing IL-6 release from mast cells, CUR was more effective in reducing TNF-α release in Caco-2, whereas BOS was more effective in reducing IL-8 release in PBMC. The reduction in IL-8 release from PBMC confirmed the results obtained by Larmonier and colleagues, who observed a similar activity in neutrophils, even if in our model we obtained different results on IL-1β [17]. Moreover, the increase in IL-10 in CUR-treated PBMC is consistent with the results obtained by McCann and collaborators [15].

In the light of these data, we assume that the modulation of the biosynthesis and release of cytokines, although important and significant, cannot actually be considered as the main mechanism of action of these phytotherapics in IBDs.

The antioxidant properties of BOS, rather than its anti-inflammatory effects, may better explain the protective effect of this extract in intestinal inflammation and could be, partly at least, marked as a specific mechanism of action, as previously reported [20,21,26]. CUR was not able to protect Caco-2 cells from H2O2-induced ROS accumulation, even if turmeric is thought to possess an important radical scavenging activity [14].

The inhibition of leukocytes infiltration, along with permeability maintenance and cytokine and ROS level reduction, observed with CUR and BOS treatment, revealed the beneficial effect of these phytotherapics on intestinal inflammation, which occurs through multiple mechanisms of action. Moreover, comparing our findings with previously reported data [26] and preliminary investigations conducted in our laboratories (data not shown), we observed no statistically significant difference between the activity of BOS and CUR compared to of acetyl-11-keto-β-boswellic acid (AKBA) and curcumin (the main constituents of boswellia and turmeric, respectively). This confirms that using the whole herbal phytocomplex, which may have an high content of AKBA and curcumin, can lead to similar therapeutic effects, but may that this may also be achieved by administrating a lower concentration of active principles.

4. Materials and Methods

4.1. Extracts preparation and chemical analysis

A commercial dry extract of Curcuma longa L. rhizome, standardized to contain 50% curcumin was bought in a pharmacy in Siena. Curcumin and curcuminoids content were quantified according to the spectrophotometric method described in the European Pharmacopoeia (Ph. Eur. 9th) [7].

The dry extract of Boswellia serrata Roxb. gum resin, standardized to contain 65% boswellic acids was gently provided by Prof Monica Montopoli, University of Padua.

Experiments were conducted in triplicate. All the chemicals and solvents were purchased from Sigma-Aldrich, Milan.
4.1. Curcumin quantification

60 ml of glacial acetic acid were added to 100 mg of CUR and the solution was heated at 90 °C for 60 minutes under reflux. 2 g of boric acid and 2 g of oxalic acid were added and the solution was heated at 90 °C for 10 minutes under reflux.

The solution was cooled to room temperature and volume adjusted to 100 ml with glacial acetic acid. 1 ml of the solution was diluted to 50 ml with glacial acetic acid and absorbance recorded at 530 nm using a spectrophotometer SAFAS UV MC2. Plastic cuvettes (1 cm optical path length) were used.

Curcumin content was calculated according to Ph. Eur. 9th as follows:

\[ \text{Curcumin \%} = \left( \frac{\text{absorbance} \times 0.426}{\text{weight (g)} \times \text{dilution factor}} \right) \]

4.1.2. Total triterpenes quantification

Boswellic acids are correctly quantified by HPLC, but in this work, considering the recent analysis performed at University of Padua [26], we confirmed the declared content of boswellic acids using the rapid colorimetric assay published by Fan and He (2006) for total triterpenes [28].

BOS was used as a 10 mg/ml ethanolic solution (96% V/V). 10 µl of the sample solution were added to 190 µl glacial acetic acid. 300 µl of a 5% m/V vanillin in glacial acetic acid solution were added and mixed for 30 seconds, before adding 1 ml of concentrated perchloric acid.

The mixture was heated at 70 °C for 40 minutes and, after cooling, the volume was adjusted to 5 ml.

Absorbance was recorded at 548 nm and quantification of total triterpenes in the extract calculated according to the calibration curve obtained using analytical grade ursolic acid.

4.2. Cell cultures

4.2.1. Peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMC) were isolated from blood obtained from 3 different healthy volunteers by density gradient centrifugation, using Histopaque®-1077 (Sigma-Aldrich, Italy) [29]. Briefly, Histopaque and freshly collected heparinized blood were added in a 15 ml tube (ratio 3:5) and spinned at 325 G for 15 minutes. Lymphomonocytes ring was recovered in a 15 ml tube and diluted to volume with saline solution. Up to three wash were performed by spinning at 240 G for 10 minutes, removing supernatant, resuspending cell pellet and diluting to volume with saline solution. PBMC were suspended in a culture medium consisting of RPMI 1460 medium with 1% L-glutamine and 1% penicillin/streptomycin solution. Cell counting was performed using a hemocytometer by Trypan blue staining and the PBMC were used immediately for experiments.

Incubation times were conducted at 37 °C, with 5% CO₂.

4.2.2. Mast cells

HMC-1.1 were used for experiments on mast cells [30]. An aliquot of HMC-1.1 cells had previously been generously gifted by Dr Butterfield to PTKS allowing us to conduct in vitro mast cell investigations. Cells were cultured in IMDM medium (Gibco, UK) supplemented with 10% calf serum (Hyclone, Logan, Utah), using 162 cm² cell culture flasks (Corning Incorporated, Costar®, USA) and incubated at 37 °C with 5% of CO₂. Cells were splitted and collected in 6 wells culture plates (Corning Incorporated, Costar®, USA) at a density of 1 x 10⁶ cells/ml. Each well contained 3 ml of cell suspension. The HMC-1.1 cells were maintained in IMDM medium, supplemented with 10% calf serum, plus alpha thioglycerol (Sigma, UK). Cell counting was performed using Countess II Life Technologies Cell Counter (Countess™ Invitrogen, USA), by Trypan blue staining.

4.2.3. Intestinal epithelium cells
Caco-2 cells were used as a stable in vitro model for the intestinal epithelium [31]. Cells were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 1% glutamine and 1% penicillin/streptomycin antibiotic (Sigma-Aldrich, Italy). Cells were maintained under a humidified atmosphere of 5% CO₂ in air, at 37 °C [25,32].

4.3. Evaluation of the anti-inflammatory activity

An in vitro inflammation model was set up for each cell line.

Samples were solubilized in ethanol 85% V/V (Sigma-Aldrich, Italy) and then diluted in cell culture medium at the concentration of 100 ng/ml, 1 µg/ml and 10 µg/ml, according to preliminary conducted cell viability tests (data not shown).

PBMC (1x10⁶ cells/ml) were seeded in 24 well plates and stimulated with LPS from Gram – (from Salmonella enteridis, Sigma-Aldrich, Italy) at a concentration of 200 ng/ml.

HMC-1.1 (1x10⁶ cells/ml) were seeded in 24 well plates and stimulated with PMA (Sigma-Aldrich, Italy) at a concentration of 50 ng/ml.

Caco-2 (4x10⁶) were seeded in 24 well plates, cultured to reach the confluence, and stimulated with LPS (500 ng/ml).

A pre-treatment of 1 hour with BOS or CUR was administered before the stimulation.

After the incubation time, samples were frozen (at -80 °C) and thawed for 3 times and the supernatants were collected for analysis.

Non-competitive sandwich ELISA kit (Biolegend e-Bioscience DX Diagnostic, Italy) were used for the dosages of TNF-α, IL-6, IL-8, IL-10, following the procedure reported in the datasheet. Absorbance was recorded at 450 nm using a SAFAS MP96 spectrophotometer.

4.4. Measurement of ROS production

ROS were quantified using 2’,7’-dichlorofluorescin-diacetate (H₂DCF-DA, Sigma-Aldrich, St Louis, MO, USA), as previously described [17]. Upon cleavage of the acetate groups by intracellular esterase and oxidation, the H₂DCF-DA is converted to the fluorescent 2’,7’-dichlorofluorescein (DCF). Briefly, the intestinal cells were seeded into 96-well plates and allowed to adhere overnight.

ROS level was measured after the exposure to BOS (1 µg/ml) or to CUR (1 µg/ml) for 24 h. Treatments were removed and H₂DCF-DA was added to obtain a final concentration of 50 µM in each well. The plate was incubated for 30 minutes at 37°C and washed with phosphate-buffered saline (PBS). DCF fluorescence intensity was measured at excitation 485 nm - emission 535 nm, using VICTOR™X3 Multilabel Plate Reader (PerkinElmer), before and after the addition of 500 µM H₂O₂ on each well.

4.5. Measurement of trans-epithelial electric resistance (TEER)

The efficiency of the barrier functions was evaluated by measuring TEER using a voltmeter [26]. Caco-2 cells were placed in Transwells (transparent PET membrane: 0.4 µm pore size; BD Falcon) as previously described [26].

The integrity of the cells were monitored by measuring the trans-epithelial electric resistance of the mono-layer at confluence from day 14° to day 20° since cells were seeded. When a stable value was reached, cells were treated with BOS (1 µg/ml) and CUR (1 µg/ml) in the appropriate wells.

After 24 hours of pre-treatment, an inflammatory stimulus (LPS 500 ng/ml) was given.

HBSS buffer was used in order to obtain from the instruments stable values of the different treatments [33,34].

TEER measurements were carried out at 3, 6, 21 and 24 hours, after the stimulation.

4.6. Adhesion assay

A simulation of inflammation-induced leukocytes infiltration on intestinal mucosa was performed developing a cell-cell adhesion assay of PBMC to Caco-2 cells, slightly modifying the
method published by Seo and colleagues [35]. Hematoxylin-eosin combination was chosen as a simple and efficient staining method [36].

Briefly, glass cover slips (VWR, Italy) were preliminary washed in ethanol (Sigma Aldrich, Italy), sterilised using a flame, covered with gelatine (Sigma-Aldrich, Italy) and incubated for 30 minutes.

Afterwards the gelatine in excess was removed and Caco-2 cells were seeded at the density of 1.5x10⁶ cells/well.

Once they reached confluence, cells were pre-treated with BOS (1 µg/ml) and CUR (1 µg/ml) for 1 hour and then exposed to LPS 500 ng/ml for 24 hour.

PBMC (3x10⁶) were co-cultured with Caco-2 for 1 hour and then washed with PBS.

Images were obtained using eclipse Ti-s (Nikon) microscope.

4.7. Statistical Analysis

All experiments were performed in triplicate in two independent repetitions (n=6).

The statistic differences between groups were determined by the analysis of the variance (one way ANOVA). Values are expressed in the range of +/- standard deviation and \( p < 0.05 \) was considered statistically significant. Graphs and calculations were performed using GraphPrism®.

5. Conclusions

Currently, a standardized protocol with universal efficacy for the treatment of chronic intestinal diseases doesn’t exist.

Corticosteroids are commonly prescribed during the acute phases of the disease. 5-aminosalicylates, immunomodulatory therapy or anti-TNF \( \alpha \) antibodies can be used in the remission phases, as an alternative or in association with corticosteroids.

All these drugs share considerable side effects and a quite low therapeutic compliance.

Therefore, the aim of this study was to evaluate new active principles with the perspective of identifying new and effective treatments for IBDs with a good safety profile.

In vitro studies and animal models largely contributed to the current comprehension of the inflammatory mechanisms at intestinal level. Those mechanisms include a loss of membrane integrity, ROS and cytokines accumulation and the hyper-activation of immune system, particularly referring to mast cells.

In the attempt to evaluate new agents for IBDs treatment, the development of inflammatory models which consider all of these elements is mandatory.

In this work, an original in vitro approach, using various human cell lines and innovative but validated methods, was proposed.

Turmeric and boswellia, which are principally characterized by the anti-inflammatory activity and by a wide and not specific anti-oxidant effect, demonstrated to be promising agents for the management of IBDs, by modulating not only the parameters indicative of dysfunction in the in vitro models used (i.e. cytokines release and ROS production), but also the ones identified in the clinical manifestation of IBDs (i.e. loss of intestinal epithelium integrity and immune cells infiltration), at concentration which can be plausibly reached at intestinal level.

Turmeric extract confirmed to possess anti-inflammatory capacity even at low concentrations and revealed itself as an extraordinary protective agent towards the intestinal epithelium integrity.

Boswellia extract showed comparable efficacy in the proposed model, exhibiting a lower anti-inflammatory effect which is counterbalanced by the strong anti-oxidant activity. Moreover, given the peculiar mechanism of action of each extract, it is likely that the association between turmeric and boswellia may act in a synergistic fashion, improving the therapeutic effectiveness.

Thus, it is hopefully that future studies will be conducted using a mixture of extracts.

This study must be considered only as a starting point in the developing process of these herbal drugs for IBDs. In that respect, it would be necessary to carry out further molecular and pharmacological investigations and to provide extracts and formulation capable of guaranteeing a good bioavailability, which is one of the main complications of these phytotherapics.
Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, Figure S1: TNF-α (a), IL-6 (b) and IL-8 (c) release induced by different concentrations of CUR and BOS in PBMC. No inflammatory effect was observed; Figure S2: TNF-α (a) and IL-6 (b) release induced by different concentrations of CUR and BOS in LPS-stimulated PBMC. No anti-inflammatory effect was observed. ** p<0.01 vs control; *** p<0.001 vs control; Figure S3: IL-6 (a) and IL-10 (b) release induced by CUR and BOS in HMC-1.1 cells. No effect was observed; Figure S4: TNF-α (a) and IL-8 (b) release induced by CUR and BOS in PMA-stimulated HMC-1.1 cells. No anti-inflammatory effect was observed. ** p<0.01 vs control; *** p<0.001 vs control; Figure S5: Effect of CUR and BOS on ROS production in Caco-2 cells; Figure S6: Effect of CUR and BOS on TEER measurements in Caco-2 cells.

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