Cape Gooseberry [Physalis peruviana L.] Calyces Ameliorate TNBS Acid-induced Colitis in Rats

Jenny Castro,† Yanet Ocampo,† Luis Franco

Biological Evaluation of Promising Substances Group, Department of Pharmaceutical Sciences, University of Cartagena, Cartagena, Colombia

†Both authors contributed equally to this work, as first authors.

Corresponding author: Dr Luis A Franco, PhD, Biological Evaluation of Promissoy Substances Group, Department of Pharmaceutical Sciences, University of Cartagena, 130015 Cartagena, Colombia. Tel: 57 5 6699771; fax: 57 5 66 98 278; Email: lfrancoo@unicartagena.edu.co

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Abstract

Background and Aims: Physalis peruviana [cape gooseberry] is highly appreciated for its commercial value. The Colombian ecotype is in great demand in the international market, particularly for the unique morphological characteristics of the calyx, which has extended use as a traditional herbal remedy in Colombia because of its anti-inflammatory properties. In this work, the anti-inflammatory activity of the total ethereal extract of Physalis peruviana calyces was evaluated in preventive and therapeutic protocols in a TNBS acid-induced colitis rat model.

Methods: Colitis was induced by intrarectal administration of TNBS. An evaluation of macroscopic and histopathological parameters in colonic tissue was performed, along with the determination of myeloperoxidase enzyme activity, cytokine levels and gene expression. Additionally, effects on nitric oxide release by lipopolysaccharide [LPS]-stimulated RAW264.7 macrophages and the scavenging activity of DPPH and ABTS free radicals were determined.

Results: The treatment with the Physalis peruviana extract produced a significant improvement in the colonic tissue at both macroscopic and histological levels. IL-1β and TNF-α production was reduced by the extract in both experimental approaches. The groups treated with Physalis peruviana showed a tendency to MUC2 up-regulation and down-regulation of COX-2, iNOS, NLRP3, IL-1β, IL-6 and IL-10 expression. Nitric oxide release in RAW264.7 macrophages was significantly inhibited.

Conclusions: The Physalis peruviana extract showed intestinal anti-inflammatory activity in the TNBS-induced colitis model, placing this species’ calyx, a natural derivative, as a promising source of metabolites that could be used in treatment for inflammatory bowel disease.

Keywords: Physalis peruviana; inflammatory bowel disease; IBD; TNBS

1. Introduction

Inflammatory bowel disease [IBD] is a chronic inflammatory disorder of the gastrointestinal tract that includes Crohn’s disease, ulcerative colitis and indeterminate colitis.1, 2 Although IBD aetiology is still not clear, it is known that its appearance implies the interaction of genetic and environmental factors, along with alterations in the immune response of the host.1, 4 It is characterized by the infiltration of lymphocytes, macrophages, neutrophils and dendritic cells capable of destabilizing the integrity of the mucosa, generating histological lesions through the synthesis and liberation of a variety of pro-inflammatory...
mediators. These include reactive oxygen species, eicosanoids, platelet activation factors, nitrogen metabolites and cytokines, which actively contribute to the pathogenic cascade that starts and maintains the chronic inflammatory response in the gut. There is no cure for this pathology, partially because the triggering factors have not been properly identified. Therefore, the therapeutic strategies have aimed to interfere at different stages of the inflammatory process and regulate the overactive immune system. Nevertheless, these therapeutic approaches are limited by their low efficacy and multiple secondary effects. Therefore, the development of safer and more effective therapeutic alternatives to treat this disease has become a priority. In this sense, plants may be a promising source of new therapeutic agents for the treatment of IBD. Several studies report plant extracts with anti-inflammatory activity, produced through the modulation of various inflammatory mediators, including interleukin (IL)-1β, IL-6, IL-10, tumour necrosis factor alpha [TNF-α], nitric oxide [NO] and prostaglandin E2 [PGE-2]. Some of these extracts are used clinically because of their beneficial effects.

Physalis peruviana. L. [Solanaeace], native of the South American Andes, is one of the most known species of this genus. This plant grows in areas about 2200 m above sea level, and is currently found in almost every tropical and some subtropical meadows, including in Malaysia, China and the Caribbean. Most of the organs from this species—for example, leaves, stems and roots—are widely employed in folk medicine because of their diuretic, antiseptic, antifungal, antibacterial, anti-carcinogenic, antimalarial and anti-inflammatory effects. Regarding the study of the calyces of Physalis peruviana, there are only a few reports of its anti-inflammatory activity, and to our knowledge, this is the first work in which this plant organ is studied using an animal IBD model. In this study, the protective and therapeutic effects of the total ethereal extract of the calyces of Physalis peruviana L. in a TNBS-acid-induced colitis model in rats was evaluated. Colonic tissue obtained from the animals was submitted to macroscopic and histological analysis. Myeloperoxidase [MPO] enzyme activity was determined, as well as the levels of TNF-α, INF-γ, IL-1β, IL-4, IL-6 and IL-10; and the differential expression of the COX-2, iNOS, MUC2, NLRP3, IL-1β, IL-6, IL-10 and IL-17 genes. In addition, the effect of the extract of Physalis peruviana on the NO production was evaluated in vitro on RAW264.7 macrophages, and its scavenging activity was measured using DPPH and ABTS assays.

2. Materials and Methods
2.1. Reagents
Dulbecco’s Modified Eagle Medium [DMEM], penicillin-streptomycin, trypan blue, lipopolysaccharide from E. coli [LPS], N-[3(aminomethyl)phenyl]methyl) ethanimidamide dihydrochloride [1400W], sodium nitrite, N-[1-naphthyl] ethylenediamine dihydrochloride, sulfanilamide, haematoxylin, eosin, trinitrobenzenesulfonic acid [TNBS], 2,2-dipheynl-1-picyrylhydraizyl [DPPH], 6-hydroxy 2,5,7,8-tetramethylchroman-2-carboxylic acid [Trolox], 2,2’-azinobis-[3 ethylbenzothiazoline-6-sulfonic acid] [ABTS] and potassium persulfate [K₃S₂O₈] were purchased from Sigma Aldrich [St Louis, MO, USA]. Bromide of 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium [MTT] was obtained from Calbiochem® [San Diego, CA, USA], Polyvinylpyrrolidone K-25 [PVP K-25], petroleum ether, chloroform and ethanol were acquired from Merck; fetal bovine serum [FBS] from GIBCO [Gaithersburg, MD, USA], dimethylsulfoxide from Carlo Erba, diazepam from Carlon S.A [Bogotá, Colombia] and ketamine from Lab Biosano [Santiago de Chile, Chile]. Macrophages RAW264.7 were acquired from the American Type Culture Collection, ATCC [Manassas, VA, USA].

2.2. Animals
Female Wistar rats and Institute for Cancer Research [ICR] mice [6–8 weeks of age] were obtained from the Instituto Nacional de Salud de Colombia. They were kept in a stress-free and controlled environment, at 22 ± 3°C and 70% ± 5% relative humidity, under 12-h cycles of light and darkness. Food and water were provided ad libitum. All experiments were designed and conducted in accordance with the guidelines of the Ethics Committee of the University of Cartagena [Minutes of October 23 of 2010] and the European Union regulations [CEC council 86/809].

2.3. Extract preparation
Physalis peruviana calyces were collected at La Mesa, Cundinamarca, and a voucher specimen was sent to the Herbario Nacional Colombiano of the National University of Colombia for its identification; it was filed under the code COLS12200. Plant material [2 kg] was dried in an airflow oven at a constant temperature of 40°C. Subsequently it was ground and extracted by percolation with petroleum ether until exhaustion, and concentrated on a rotary evaporator with reduced pressure and controlled temperature [35-45°C] to obtain 200g of total extract. Given the low solubility of Physalis peruviana extract in aqueous solution, it was necessary to co-precipitate it with PVP K-25 in a extract: PVP [1:4 w/w] ratio, and was it was solubilised in phosphate buffer saline [PBS] for administration. Qualitative identification of alkaloids, flavonoids, nafto and/or anthraquinones, tannins, saponins, terpenoids, coumarins, terpene and lactones was performed by the protocols established by Sanabria-Galindo et al. 1997, and the identification of glycosides according to the protocol of Tiwari et al. 2011.

2.4. Acute toxicity
To define the adequate doses for in vivo anti-inflammatory activity assays, the acute toxicity of the Physalis peruviana extract was evaluated for a 24-h period. Lethal dose 50 [LD₅₀] was determined following the protocol described by Al-Sultan et al. 2006, with some modifications. Briefly, ICR mice were randomly distributed into seven groups of six animals each. Doses between 300 and 450 mg/kg of Physalis peruviana extract, administered intraperitoneally [i.p.], were selected according to previous studies. For histological analysis, liver and kidney samples were preserved in buffered formalin and stained with haematoxylin and eosin.

2.5. Induction of colitis and treatments
Colitis was induced employing the method described by Morris et al. 1989, with some modifications. Briefly, Wistar rats were not fed for 12h, and were anaesthetised with a mixture of ketamine [100mg/kg] and diazepam [5 mg/kg] i.p. Subsequently, 0.25 ml of an ethanolic solution of TNBS [40mg/ml] was instilled rectally using a cannula that was introduced to 8cm. A healthy control group was included, which was instilled with saline solution. The different doses of the Physalis peruviana extract used were calculated at about 1/3 and 1/6 of its LD₅₀. The anti-inflammatory effect of the extract was studied using two different protocols [Figure 1]. In the first approach, the protective effect was evaluated. The rats received 125 mg/kg i.p. at 48, 24 and 2h before colitis induction as well as 24h thereafter. In the second approach, the therapeutic effect was evaluated administering a dose of 62.5 mg/kg i.p. for 15 days and the colitis induction was performed from the first day of treatment. The healthy control and TNBS groups were treated with sterile PBS as a vehicle. Animal weight and food consumption were monitored on a daily basis. At the end of each experiment the animals were sacrificed by cervical dislocation. The colon was extracted, cleaned, weighed and macroscopically analysed, determining a colonic
tissue damage score using the scale of Bobin-Dubigeon et al. 2001. A colonic tissue sample was preserved in buffered formalin, embedded in paraffin, cut into 5-μm sections and stained with haematoxylin and eosin or Periodic Acid-Schiff (PAS) for histopathological analysis. The rest of the colon was kept for subsequent determination of the MPO activity, cytokine levels and gene expression analysis through real-time polymerase chain reaction (RT-PCR).

2.6. MPO activity on colonic tissue
The enzyme activity was measured according to the technique described by Castro et al. 2014, and the results were expressed as MPO units per gram of wet tissue; one unit of MPO activity was defined as that degrading 1 mmol of hydrogen peroxide/min at 25°C.

![Figure 1. Experimental design of TNBS acid-induced colitis. Wistar rats (n = 10 per group) were rectally instilled with 0.25 ml of TNBS ethanol solution (40 mg/ml) and Physalis peruviana extract (125 mg/kg/day—preventive approach or 62.5 mg/kg/day—therapeutic approach) or phosphate buffer saline (PBS) intraperitoneally (i.p.). In both assays a sham group was included.](image)

![Figure 2. Body weight changes following the TNBS acid colitis induction. (A) Preventive approach (Physalis peruviana [P. p] extract 125 mg/kg/day [i.p.]). (B) Therapeutic approach (Physalis peruviana extract 62.5 mg/kg/day i.p.). The results represent the daily changes in body weight in Wistar rats per group. Body weight change was calculated by dividing body weight on the specified day by body weight at Day 0 (starting body weight) and expressed in percentage ± standard error of the mean (SEM) (n = 10). *P < 0.05; **P < 0.01; ***P < 0.001 analysis of variance (ANOVA), statistically significant compared with TNBS group.](image)
2.7. Cytokine levels on colonic tissue
The colonic tissue was homogenised in a Greenburger buffer supplemented with protease inhibitors [Complete Mini EDTA free Roche], sonicated for 10 s and centrifuged at 10000 rpm at a temperature of 4°C for 20 min. The levels of TNF-α, INF-γ, IL-1β, IL-4, IL-6 and IL-10 were quantified on the obtained supernatants using commercial enzyme-linked immunosorbent assay [ELISA] kits [Invitrogen, Carlsbad, CA, USA and R&D Systems, Minneapolis, MN, USA for IL-1β], following the manufacturer’s protocol. The results of cytokine release were normalized by the protein content, which was quantified by the Bradford method, using a standard commercial kit [Biorad 500-0206].

2.8. Gene expression on colonic tissue by RT-PCR
Extraction of total mRNA from the tissue obtained in the therapeutic experiment was performed with a commercial kit [QIAGEN RNaseasy® 74106], following the instructions provided by the manufacturer and quantified in a Nanodrop 2000c [Thermo Scientific]. The cDNA was synthesised using QIAGEN Quantitect® 205313 kit and amplified with power SYBR® Green PCR master mix [Applied Biosystems] and specific primers [Eurofins Genomics] in a RT-PCR LightCycler® 96 System [Roche]. The mRNA relative expression of COX-2, iNOS, MUC2, NLRP3, IL-1β, IL-6, IL-10 and IL-17 was calculated using the ΔΔCt method and normalised with glyceraldehyde-3-phosphate dehydrogenase [GAPDH] as a housekeeping gene.

2.9. NO production by RAW264.7 macrophages
RAW264.7 macrophages were maintained on DMEM supplemented with 10% FBS and penicillin-streptomycin; at 37°C in an atmosphere of 5% CO₂ and 100% humidity. The cell viability was evaluated employing an MTT colorimetric assay. To determine the effect of Physalis peruviana extract on NO release, RAW264.7 macrophages [2 x 10⁶ cells/ml] were incubated on 96-well plates with non-toxic concentrations of the sample, or the 1400W used as a control. After 30 min, cells were stimulated with LPS [1 µg/ml] and incubated again for 24 h under the previously described conditions. The Griess reagent was added to the obtained supernatants in a 50:50 ratio and the optical density [OD₅₅₀] was determined in a Multiscan EX Thermo microplate reader. Nitrite concentration on the supernatants was established using a standard curve of NaNO₂. In each assay a non-stimulated control group and a group treated with LPS alone were included. Additionally, the NO scavenging effect of the extract was evaluated using the method previously described by Castro et al. 2014.

2.10. Antioxidant activity
The scavenging activity of the extract of Physalis peruviana on the DPPH and ABTS radicals was determined spectrophotometrically, employing protocols adapted for microplate reading. For the DPPH, 75 µl of the extract were added to 150 µl of a DPPH solution [100 µg/ml]. This mixture was incubated at 25°C for 30 min and the OD₅₅₀ was measured. To determine the effect on the ABTS radical, 10 µl of the extract were mixed with 190 µl of an ABTS ethanolic solution, produced by the reaction of ABTS [3.5 mM] with potassium persulfate [1.25 mM], incubated in the dark for 16 h at room temperature, and diluted with ethanol to an absorbance of 0.7±0.1 at 405 nm. The final mixture was incubated at 23°C for 5 min and the OD₄₅₀ was determined. In both assays, vehicle and Trolox were used as negative and positive controls, respectively. The results are shown as µmol Trolox/g of calyces.

2.11. Statistical analysis
The results are expressed as the mean ± standard error of the mean [SEM] and analysed employing a one-way analysis of variance [ANOVA], followed by Tukey post hoc. Values of P < 0.05 were considered significant. The LD₅₀ was calculated using the Probit method.

3. RESULTS
3.1. Physalis peruviana extract
The preliminary phytochemical analysis of the total ethereal extract from calyces of Physalis peruviana permitted the identification of the presence of flavonoids, terpenoids and glycosides. With regard to its toxicity, the estimated DL₅₀ was 345.49 mg/kg i.p. [confidence...
3.2. *Physalis peruviana* anti-inflammatory effect on rats with colitis

The administration of TNBS significantly decreased food consumption and a consequent weight loss was observed, just as in human clinical pathology. In both approaches, the animals of the TNBS and *Physalis peruviana* groups suffered a drastic weight loss compared with the healthy control group, and significant differences were not observed between TNBS and *Physalis peruviana* groups [Figure 2]. In both experimental approaches, TNBS produced damage to the intestinal mucosa of the rats, with transmural involvement, ulcerations, oedema and tissue thickening [Figure 3B, E], whereas the healthy groups maintained their normal structure [Figure 3A, D].

The treatment with the *Physalis peruviana* extract [Figure 3C, F] produced a significant improvement in the colonic tissue, decreasing the damage score, extent of damaged areas and weight/length ratio of the tissue [Table 1].

Histologically, the animals from the healthy control groups showed typical characteristics of a normal structure [Figure 4A, E], whereas the TNBS group showed an intense transmural interruption, extensive ulceration, inflammation, oedema and massive infiltration of neutrophils, mainly in the mucosa [Figure 4B, F]. In the preventive approach, the animals treated with *Physalis peruviana* extract showed less oedema and neutrophil infiltration as well as a restoration of the mucosa, submucosa and muscular layers [Figure 4C, D]. The therapeutic approach, on the other hand, showed restoration limited to the mucosa [Figure 4G, H], in contrast with the TNBS group that presented crypt loss and goblet cells depletion [Figure 5].

3.3. MPO activity

The MPO enzyme is mainly produced by neutrophils. The results of the *Physalis peruviana* extract evaluation showed a slight reduction in MPO activity induced by TNBS in both approaches [Figure 6], suggesting a decreased infiltration of neutrophils in the colonic tissue, which is consistent with what was observed in the histopathological analysis [Figure 4].

3.4. Cytokine levels

TNBS administration produced a reduction of IL-4 levels and an increase of TNF-α, IFN-γ, IL-1β, IL-6 and IL-10 levels in the colonic tissue of the animals. *Physalis peruviana* extract significantly reduced the levels of the pro-inflammatory cytokines TNF-α and IL-1β in the preventive and therapeutic experiments, whereas the levels of IL-10, IL-6, IL-4 and IFN-γ did not change [Figure 7].

3.5. RT-PCR

The TNBS-induced colitis produced an increase in the expression of COX-2, iNOS, NLRP3, IL-1β, IL-6, IL-10 and IL-17 genes, while reducing the expression of MUC2, a protein involved in the epithelial integrity. In the groups treated with *Physalis peruviana*, a tendency to up-regulate MUC2 and down-regulate COX-2, iNOS, NLRP3, IL-1β, IL-6 and IL-10 expression was observed, without statistical differences [Figure 8]. This tendency of the suppression of the genes involved in the inflammatory response can be responsible for the anti-inflammatory activity of the *Physalis peruviana* extract on the TNBS-induced colitis model.

3.6. *Physalis peruviana* effect on NO production

The extract of *Physalis peruviana* did not show toxicity on RAW264.7 macrophages at concentrations equal or lower than 30 µg/ml [data not shown]. This extract significantly inhibited NO release from the macrophages with an inhibitory concentration [IC50] of 29.64 µg/ml [95% confidence intervals, 25.11–35.27 µg/ml] [Figure 9]. This reduction of the NO levels in the culture medium of the macrophages treated with *Physalis peruviana* extract is not related to a NO radical scavenging effect [data not shown].

3.7. Antioxidant activity

*Physalis peruviana* extract did not produce a significant scavenging activity of the DPPH and ABTS radicals with IC50 greater than 500 µg/ml in both methods, corresponding to values of 0.764 ± 0.0076 and 1.645 ± 0.0646 nmol Trolox/g extract, respectively.

4. Discussion

The calyx that envelops the *Physalis peruviana* fruit is formed by sepals or modified leaves that contribute to the development and maturation of the fruit, protecting it from insects, birds, diseases and adverse climatic situations. The anti-inflammatory activity showed by the extract of the *Physalis peruviana* calyces in the IBD model might be related to the presence of flavonoids, terpenoids and glycosides, metabolites found in this organ. These have been reported to affect the production of TNF-α, IL-1β and NO in studies related to IBD. These compounds have also been found in other parts of the plant such as the leaves, fruit and roots.

The extract obtained from the *Physalis peruviana* calyces showed a toxic effect, higher than that observed for leaf extracts in other

Table 1. Effect of *Physalis peruviana* [Pp] extract on the damage score, damaged area and weight/length ratio, in TNBS acid-induced colitis. Sham and TNBS groups were treated with phosphate buffer saline [PBS] in both approaches. *Pp* was administered at 125 mg/kg/day intraperitoneally [i.p.], [preventive approach] and 62.5 mg/kg/day i.p. [therapeutic approach].

<table>
<thead>
<tr>
<th>Group</th>
<th>Damage score [0–10]</th>
<th>Damaged area [mm²]</th>
<th>Weight/length ratio [mg/cm]</th>
</tr>
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<tbody>
<tr>
<td>Preventive: 5 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>0.0***</td>
<td>0.0***</td>
<td>69.4 ± 4.2***</td>
</tr>
<tr>
<td>TNBS</td>
<td>8.1 ± 0.6</td>
<td>627.4 ± 69.5</td>
<td>133.7 ± 10.1</td>
</tr>
<tr>
<td>Pp</td>
<td>0.0***</td>
<td>0.0***</td>
<td>76.7 ± 5.3***</td>
</tr>
<tr>
<td>TNBS + Pp</td>
<td>3.4 ± 0.6***</td>
<td>305.3 ± 76.7***</td>
<td>89.4 ± 4.5**</td>
</tr>
<tr>
<td>Therapeutic: 15 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
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<td>0.0***</td>
<td>62.6 ± 2.1***</td>
</tr>
<tr>
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<td>450.6 ± 101.87</td>
</tr>
<tr>
<td>TNBS + Pp</td>
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<td>630.02 ± 41.35**</td>
<td>192.0 ± 9.6†</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard error of the mean [SEM] [n = 10]. *P < 0.05; **P < 0.01; ***P < 0.001 analysis of variance [ANOVA], statistically significant compared with TNBS.
Physalis Peruviana Ameliorates TNBS Acid-induced Colitis

Studies. This elevated toxicity is consistent with the protective function of the plant calyces to safeguard the fruit, and suggests the presence of potent bioactive metabolites.

The rectal instillation of 2,4,6-trinitrobenzene sulfonic acid [TNBS] and ethanol is a chemical model widely employed to produce chronic colonic inflammation with morphological and histological alterations that resemble IBD in humans. Physalis peruviana showed a strong anti-inflammatory effect in rats with TNBS-induced colitis, in both the preventive and therapeutics approaches. Macroscopically, the damage score, the extent of damaged areas and the weight/length ratio were reduced. This last parameter is considered an important and dependable tissue damage index. It shows increases in severe intestinal inflammation, since oedema and tissue thickening produce a reduction in colon length, resulting from the

Figure 4 Representative histological colonic tissue sections from groups included in the colitis experiment; stained with haematoxylin and eosin. Preventive approach: [A] sham, [B] TNBS acid and [C] TNBS + Physalis peruviana [P. p] extract [125 mg/kg/day intraperitoneally] [i.p.]. Therapeutic approach: [E] sham, [F] TNBS and [G] TNBS + Physalis peruviana extract [62.5 mg/kg/day i.p.]. A blinded pathologist analysed the sections. Original zoom x10. The damage score was assigned using the assessment scale described by Stucchi et al. 2000 with some modifications [D and H]. The scale presents a score between 0 and 4 [0, none; 1, faint; 2, slight; 3, moderate; 4, severe]. The results represent the mean obtained from the addition of the scores for each animal ± standard error of the mean [SEM] [n = 10]. ***P < 0.001 analysis of variance [ANOVA], statistically significant compared with TNBS acid.
Figure 5. Microscopic images of periodic acid Schiff (PAS)-stained colon sections from the groups included in the therapeutic approach. [A and B] sham, [C and D] TNBS acid and [E and F] TNBS + Physalis peruviana extract (62.5 mg/kg/day intraperitoneally [i.p.]). A blinded pathologist analysed the sections. Original zoom x10 [A, C and E] and zoom x20 [B, D and F].

Figure 6. Myeloperoxidase (MPO) enzyme activity in colonic segments obtained from the groups included in the TNBS acid-induced colitis experiment. The results represent the mean ± standard error of the mean [SEM] [n = 10]. ***P < 0.001 analysis of variance (ANOVA), statistically significant compared with TNBS acid.
Physalis peruviana ameliorates TNBS acid-induced colitis

contraction of the muscular layer of the intestinal wall, as well as the fibrosis presented as a consequence of the constant scarring process in the intestinal mucosa. This beneficial effect was corroborated histologically.

MPO is an enzyme widely distributed throughout the body. Its principal source is the azurophilic granules of the neutrophils. In IBD, there is an elevated activity of this enzyme as a result of the recruitment of neutrophils in the intestinal mucosa from chemokines like CXCL5; therefore, MPO is considered an important biomarker of the infiltration of neutrophils in colonic tissue.

The anti-inflammatory activity shown by the Physalis peruviana extract appears to be partially related to its capacity to reduce the infiltration of neutrophils in the colonic tissue, which was also clearly observed in the histopathological results.

In IBD, macrophages, dendritic cells and T lymphocytes secrete cytokines uncontrollably. These promote the production of reactive oxygen species, NO, prostaglandins, leukotrienes and other mediators with a consequent loss of the homeostasis that leads to a sustained inflammatory response. Similarly, the TNBS administration to the animals produces an increase in TNF-α, IL-1β, IFN-γ, IL-6, IL-10, IL-12, IL-23 and IL-17 levels, while reducing the levels of IL-4 in the colonic tissue. Physalis peruviana extract was able to significantly reduce the levels of TNF-α and IL-1β in the colonic tissue. These cytokines share several pro-inflammatory

Figure 7. Physalis peruviana [P. p] extract effect on TNF-α, INF-γ, IL-1β, IL-4, IL-6 and IL-10 levels in colonic tissue. Tissue lysates were analysed using enzyme-linked immunosorbent assay [ELISA]. The results represent the mean ± standard error of the mean [SEM] [n = 10]. *P < 0.05; **P < 0.01; ***P < 0.001 analysis of variance [ANOVA], statistically significant compared with TNBS acid.
TNF-α induces the release of pro-inflammatory cytokines such as IL-1β. In this sense, it is possible that the reduction of IL-1β levels produced by the Physalis peruviana extract may be related to the inhibition of TNF-α release.

The benefits from the regulation of TNF-α have been proven by the use of anti-TNF-α drugs, which are effective in reducing the inflammation but have elevated costs and multiple side effects for the patients. As a result, the anti-inflammatory activity of the Physalis peruviana extract at macroscopic, histological and biochemical levels, appearing to be closely related to the reduction of the TNF-α production, shows Physalis peruviana as a promising source for obtaining new bioactive compounds for the regulation of IBD.

The activation of the macrophages RAW264.7 with LPS triggers characteristic events of acute and chronic inflammatory processes, promoting the synthesis of inflammatory mediators including the NO radical. Physalis peruviana extract reduced the NO release in RAW264.7 macrophages, and showed a trend to down-regulate the inducible nitric oxide synthase (iNOS) expression in the colonic...
tissue. Thus, NO seems to be another important target of the anti-inflammatory effect produced by the calyces of this species. The inhibition of the production of this radical has become the object of research aimed at the development of anti-inflammatory drugs for the treatment of IBD, given the association found between NO overproduction and severe damage to the mucosa in this pathology.\textsuperscript{57,58,59}

The reactive oxygen species are metabolites associated with the initiation and progression of IBD. Control of their synthesis is considered an important target in the search for new therapeutic agents.\textsuperscript{60} Gironés-Vilaplana \textit{et al}., 2014, reported a hydro-alcoholic extract with DPPH and ABTS radical scavenging activity obtained from the calyces of \textit{Physalis peruviana}.\textsuperscript{61} In our study, the total ethereal extract obtained from the calyces of this species did not show a significant scavenging activity on these free radicals, which suggest that the metabolites responsible for this activity are hydrophilic, being more affined to polar phases like the one used by Gironés-Vilaplana. Our results show the strong anti-inflammatory activity displayed by \textit{Physalis peruviana} extract is not related to free radical scavenging activity.

In conclusion, the total ethereal extract associated with the calyces of \textit{Physalis peruviana} significantly reduces intestinal inflammation induced by TNBS in rats. This activity seems to be related to the reduction of the migration of neutrophils into the colonic tissue, and restoration of the intestinal mucus layer, as well as the decrease of TNF-\textalpha, IL-1\textbeta and NO levels, considered crucial targets in IBD. Additional research is needed to increase knowledge about the mechanisms implicated and elucidate the compounds responsible for the anti-inflammatory effect shown by \textit{Physalis peruviana}.

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**Conflict of Interest**

The authors declare no conflicts of interest.

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**Author Contributions**

LF contributed to the concept and design of the study and, together with JC and YO, performed the experiments. All the authors contributed to the analysis and interpretation of the data. JC wrote the manuscript; YO and LF reviewed and approved the final draft.

**References**


