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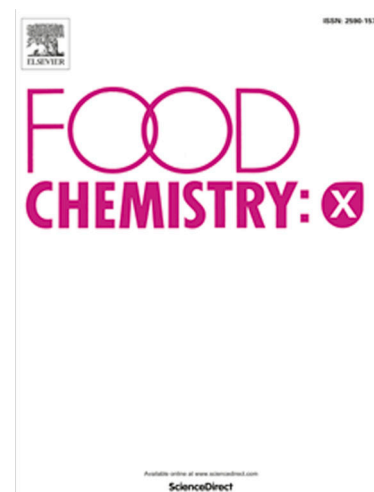
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OXYACANTHA) LEAVES AND FLOWERS ATTENUATES COLITIS IN RATS BY
REDUCING INFLAMMATION AND OXIDATIVE STRESS

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28

29 ABSTRACT

30 The purpose of the study was to determine the effects of a tea from the leaves and flowers of
31 *Crataegus oxyacantha* in rats with colitis. Colitis was induced by administration of 2,4,6-
32 trinitrobenzene sulfonic acid. Hawthorn tea (HT) (100 mg/kg) was given via gavage for 21 days and
33 the mesalamine drug (100 mg/kg) was administrated during the period of disease onset. HT was
34 rich in total phenolic compounds (16.5%), flavonoids (1.8%), and proanthocyanidins (1.5%);
35 vitexin-2-O-rhamnoside was the main compound detected. Mesalamine and the HT diminished the
36 length of the lesions formed in the colon, in addition to reducing the levels of myeloperoxidase and
37 interleukin-1 β . Mesalamine was able to significantly reverse the body weight loss, while HT
38 improved the activity of glutathione reductase and catalase. Histological scoring was not changed
39 by the interventions, but it was highly correlated with the necrotic area. HT given at 100 mg/kg can
40 be effective against colitis.

41

42 **Keywords:** *Crataegus oxyacantha*; polyphenol; vitexin-2-O-rhamnoside; inflammatory bowel
43 diseases; colon.

44

45 ABBREVIATIONS

46 CAT - Catalase

47 CD - Crohn's disease

48 DAD - Diode array detection

49 DAI - Disease Activity Index

50 DSS - Dextran sodium sulfate

51 ELISA - Enzyme-linked immunosorbent assay

- 52 ESI - Electrospray ionization
- 53 FID - Flame ionization detector
- 54 FRAP - Ferric reducing antioxidant power
- 55 GC - Gas chromatograph
- 56 GPx - glutathione peroxidase
- 57 GR - Glutathione reductase
- 58 GSH - Glutathione
- 59 HT - Hawthorn tea
- 60 IBD - Inflammatory bowel disease
- 61 IL-1 β - Interleukin-1beta
- 62 MDA - Malondialdehyde
- 63 MPO - Myeloperoxidase
- 64 MS - Mass spectrometry
- 65 ORAC - Oxygen-radical absorbing capacity
- 66 SCFA - Short-chain fatty acid
- 67 SOD - Superoxide dismutase
- 68 TFC - Total flavonoids content
- 69 TNBS - 2,4,6-trinitrobenzene sulfonic acid
- 70 TNF- α - Tumor necrosis factor-alpha
- 71 TPC - Total polyphenols content
- 72 TPOC - Total proanthocyanidin oligomers content
- 73 UC - Ulcerative colitis
- 74 UHPLC - Ultra-high-performance liquid chromatography

75

76 **1. INTRODUCTION**

77 in the last 20 years, there has been increasing *in vitro* and *in vivo* evidence of the beneficial
78 health effects of functional foods, teas, and extracts from plants. Such alternative or complementary
79 medicine has been suggested as a new tool against non-communicable diseases, which eventually
80 may impact in the future for changes in prevention guidance and the treatment of debilitating
81 chronic illness, such as cardiovascular diseases, inflammatory bowel diseases (IBDs), and cancer
82 (Campos, 2019). However, more studies are needed in order to understand and deepen the effective
83 dosages and mechanisms behind the biological action of natural bioactive compounds. Notably, the
84 research on the utilization of the residues of plants, usually commercially discarded, such as stems,
85 leaves, and flowers, is probably one of the strands that should be more investigated within the field
86 of medicinal plants and herbs, given their great chemical and nutritional composition (Simpson et
87 al., 2019).

88 Europe has a wide range of native plants spread through its territory, and one of the most
89 common ones is hawthorn (*Crataegus spp.*), a tree belonging to the Rosaceae family (Chang et al.,
90 2002). Hawthorn is commonly used as a hedging species in England and Denmark (Billing et al.,
91 1974; Weber, 2010), and as a herbal remedy throughout the continent (Dahmer & Scott, 2010).
92 Especially, the hawthorn extracts or teas from the leaves and flowers of *Crataegus oxyacantha* and
93 *Crataegus monogyna* have been officially approved in Germany to treat patients with mild to
94 moderate chronic congestive heart failure (Nathan, 1999) due to its high content in flavonoids and
95 proanthocyanidins (Ngoc et al., 2019). It is known that flavonoids and proanthocyanidins can 1.
96 decrease the inflammatory processes, 2. act by increasing the activity of enzymes that control the
97 oxidation of the cell membrane (Panche et al., 2016; Rauf et al., 2019), and 3. possibly modulate the
98 microbiome environment by being fermented by the microbiota (Cardona et al., 2013). Despite that,
99 hawthorn has been mostly associated with beneficial effects in cardiovascular complications and
100 hypertension (Dalli et al., 2011; Walker et al., 2006), while research on their effects in intestinal
101 health and against gut diseases, like IBDs, for example, are lacking.

102 IBDs, mainly ulcerative colitis (UC) and Crohn's disease (CD), largely affect the
103 gastrointestinal tract, producing a high-grade immune-associated inflammatory and oxidative
104 process (Ungaro et al., 2017). Such diseases possess a high prevalence in Europe and are increasing
105 at a high incidence rate since the '90s in newly industrialized countries, such as Brazil and Taiwan
106 (Ng et al., 2017). Treatment options for IBDs are commonly not well accepted or tolerable for a
107 great part of patients (Horne et al., 2009; Lasa et al., 2020; Pieper et al., 2009), suggesting the need
108 for studies capable of proving new effective options. From our knowledge, four studies to date have
109 attested the positive effects of hawthorn in IBDs; however, these investigations used the fruits, not
110 the leaves or flowers, and distinct hawthorn species (Fujisawa et al., 2005; Guo et al., 2021; Liu et
111 al., 2020; Malekinejad et al., 2013). Fujisawa et al. (2005) reported that the freeze-dried aqueous
112 extract from the fruit of *Crataegi fructus* given in the drinking water prevented the inflammatory
113 outcome and high mortality of chemically-induced models of colitis (Fujisawa et al., 2005). Liu et
114 al. (2020) described that the freeze-dried hydroalcoholic extract from the fruit of *Crataegus*
115 *pinnatifida*, rich in flavonoids, inhibited the secretion of proinflammatory cytokines and alleviated
116 the increase of paracellular permeability in Caco-2 cells, therefore, indicating its protective effects
117 against epithelial barrier dysfunction (Liu et al., 2020). Such initial studies indicate the potential of
118 the hawthorn plant against IBDs.

119 No publication to date investigated the preventive effects of *Crataegus oxyacantha* or the
120 tea from its flowering tops (leaves and flower buds) in broader aspects of IBDs, such as
121 inflammation, oxidative stress, and microbiome health. Therefore, in order to understand if the
122 effects of the residual parts of this plant are extended further than its most-known cardioprotective
123 role, this study aimed to investigate the preventive implications of the administration of a
124 polyphenol-rich dry tea from the *Crataegus oxyacantha* flowering tops in a model of IBD-like
125 colitis induced in rats by 2,4,6-trinitrobenzene sulfonic acid (TNBS).

126

127 2. MATERIAL AND METHODS

128 **2.1. Chemicals**

129 Aluminum chloride hexahydrate, n-butanol, Folin-Ciocalteu reagent, gallic acid (GA),
130 quercetin (Q), and cyanidin chloride (CY) were purchased from Merck (Saint-Quentin Fallavier,
131 France). *Crataegus spp.* extract standard (R1) was acquired from HWI Group (Rülzheim, Germany)
132 and standardized at 29 mg of vitexin 2-O-rhamnoside per g of extract. Procyanidin A2, B2 and C1,
133 epicatechin, cinnamtannin A2, and isoquercetin were purchased from Phytolab (Vestenbergsgreuth,
134 Germany). 2,2'-Azobis(2-amidinopropane) dihydrochloride was purchased from Cayman Chemical
135 (Ann Arbor, MI, USA), fluorescein was purchased from Synth (Diadema, SP, Brazil) and
136 metaphosphoric acid was purchased from Vetec Quimica Fina Ltda. (Rio de Janeiro, RJ, Brazil).
137 Protein Assay Dye Reagent Concentrate was acquired from Bio-Rad Laboratories (Hercules, CA,
138 USA). Thiobarbituric acid was obtained from Merck KGaA (Darmstadt, Germany). Enzyme-linked
139 immunosorbent assay (ELISA) kits were purchased from Peprotech (Rocky Hill, NJ, United States).
140 1,1,3,3-tetraethoxypropane, 2,4,6-Tripyridyl-S-triazine, 5,5'-dithiobis-(2-nitrobenzoic acid), 6-
141 hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), bovine serum albumin, catalase
142 (CAT), glutathione (GSH), myeloperoxidase (MPO), nitro blue tetrazolium chloride, oxidized
143 glutathione, reduced nicotinamide-adenine dinucleotide phosphate, standards of short-chain fatty
144 acids (SCFAs) (2-ethylbutyric acid, acetic acid, butyric acid, and propionic acid), sodium dodecyl
145 sulfate and TNBS were all obtained from Sigma Aldrich (St. Louis, MO, USA).

146

147 **2.2. Hawthorn tea production and chemical characterization**

148 Dry hawthorn flowering tops (*Crataegus oxyacantha*) (leaves and flower buds) were
149 purchased from France Herboristerie (Lot number 55849, Noidans-Lès-Vesoul, France). The plants
150 were previously grounded using an Ika grinder (Ika-Werke GmbH, Model MF10 basic, Staufen,
151 Germany) and a “1 mm” grid. The material size distribution was determined by dry laser
152 granulometry.

153 The tea was produced according to NGOC et al. (NGOC et al., 2019). A sample of 50 g was
154 infused in 1 L of boiling water using a 'French press' Bodum® under 500 rpm magnetic stirring.
155 After 30 min, the herbal tea solution was filtered first with the Bodum® cover to remove the largest
156 particles, then with a Whatman filter paper placed in a Büchner funnel using a vacuum pump to
157 remove any residue of the solid plant. Finally, the herbal tea solution was concentrated using a
158 rotary evaporator and later freeze-dried (Cryotec Model CRIOS-80, Saint-Gély-du-Fesc, France) to
159 obtain the dry hawthorn tea (HT). The same experiment was repeated three times to get a total of 30
160 g. Lyophilized tea was stored at 4°C (Ngoc et al., 2019).

161 Total polyphenols (TPC) (mg of gallic acid equivalent/g dry weight), total flavonoids (TFC)
162 (mg of quercetin equivalent/g dry weight), and total proanthocyanidin oligomers (TPOC) (mg of
163 cyanidin equivalent/g dry weight) contents were determined according to the Folin-Ciocalteu
164 method (Singleton & Rossi, 1965), the aluminum chloride method (Lamaison & Carnat, 1990) and
165 the HCl/n-butanol assay (Porter et al., 1985), respectively. Analyses were performed in triplicate.

166 For the quantification and identification of specific phenolic compounds, an ultra-high-
167 performance liquid chromatography associated with diode array detection (UHPLC-DAD) and
168 coupled with electrospray ionization and mass spectrometry (UHPLC-ESI-MS), respectively, were
169 utilized. First, 20 mg of the dry HT was dissolved in 1 mL of water and vortexed for 2 min. The
170 resulting solution was diluted five times with water and vortexed for another 2 min prior to the
171 analysis.

172 The UHPLC-DAD system consisted of a Thermo Scientific™ Dionex™ UltiMate™ 3000
173 BioRS equipped with a WPS-3000TBRS autosampler and a TCC-3000RS column compartment set
174 at 35°C (ThermoFisher Scientific, Waltham MA, USA). The system was operated using
175 Chromeleon 7 software. A Luna® Omega polar C18 column (1.6 µm, 100 × 2.1 mm) combined
176 with a security guard ultra-cartridge was used (Phenomenex Inc., Torrance CA, USA). A binary
177 solvent system was utilized, consisting of water/formic acid (1%, v/v) as solvent A and
178 acetonitrile/formic acid (1%, v/v) as solvent B. The gradient program started with 5% B, then B was

179 increased to 100% in 30 min with a convex increase (curve 5 in Chromeleon 7). The flow rate of
180 the mobile phase was 0.4 mL/min, and the injection volume was 4 μ L. The peaks were monitored at
181 273 nm. The UV-Vis spectra of the different compounds were recorded between 200 and 550 nm
182 using the diode array detector.

183 The UHPLC-ESI-MS analysis was performed using a Synapt G2-S (Waters Corp., Milford
184 MA, USA) equipped with an ESI. The column, injection volume, flow rate, and gradient program
185 were the same as for UHPLC-DAD. A positive mode was used. The capillary voltage was set to 3
186 kV, the cone voltage was set to 30 V, and the extract or voltage was set to 3 V. The source
187 temperature was 100°C, and the desolvation temperature was 450°C. MS spectra were obtained by
188 scanning ions between 100 and 1500 m/z. The system was operated using MassLynx 4.1 software.

189 The quantification of vitexin-2-O-rhamnoside, a common and abundant flavonoid found in
190 hawthorn species (Alirezalu et al., 2018; Kumar et al., 2012), was determined by external
191 calibration, using a commercially available standard. All other components identified in the HT
192 were expressed as peak area ratio from the chromatogram, since the standards were not available.

193

194 **2.3. Animal experimentation**

195 **2.3.1. Ethics, diet, and conditions**

196 The study is in accordance with the ARRIVE guidelines and followed the guide for the care
197 and use of laboratory animals of the National Institutes of Health (NIH Publications No. 8023,
198 revised 1978), and the Brazilian National Council for Animal Experiments Control - CONCEA.
199 The Ethics Committee on the Use of Animals of the University of Campinas approved the
200 experimental protocol (number 5042-1/2018, UNICAMP).

201 Male Wistar rats (n=29) were obtained from the Multidisciplinary Center for Biological
202 Research on Laboratory Animal Science at UNICAMP and allocated in an experimentation room
203 with the temperature at controlled levels (20-22°C). Rats were submitted to a standard daily 12 h:12
204 h light-dark cycle. During the acclimatization and experimental periods, rats received a commercial

205 pelleted diet (NUVITAL CR-1, NUVITAL) and water *ad libitum*. Animals entered the experimentation
206 room at four weeks of age and were submitted to the procedures starting at seven weeks of age.

207

208 2.3.2. Intervention, colitis induction, and clinical evaluation

209 A total of 29 Wistar rats were divided in five experimental groups: *control-C* (n=5), *control*
210 *colitis-CC* (n=6), *hawthorn tea-H* (n=5), *hawthorn tea colitis-HC* (n=6) and *mesalamine colitis-MC*
211 (n=7). Groups with colitis had more animals since death may happen in the TNBS model (Antonioni
212 et al., 2016). Dry HT was diluted in distilled water and given preventively for 14 days at a dosage
213 of 100 mg/kg of body weight via gavage. The dosage chosen was based on the majority of studies
214 found in the literature (Elango et al., 2009; Elango & Devaraj, 2010; Hatipoğlu et al., 2015). The
215 intervention with HT (groups *H* and *HC*) continued until the end of the experiment, including the
216 period after colitis induction (when applicable). Other groups (*C*, *CC*, *MC*) received distilled water
217 via gavage instead.

218 Acute colitis was induced on the 14th day using TNBS (10 mg) dissolved in 250 µL of 50%
219 ethanol (v/v) (da Silva-Maia et al., 2019) and administrated via rectal in rats previously sedated
220 intraperitoneally with ketamine (75 mg/kg of body weight) and xylazine (10 mg/kg of body
221 weight). Groups without colitis induction (*C*, *H*) received saline solution (0.9%) instead of TNBS.

222 Mesalamine, a drug commonly used to treat IBDs (Lacucci et al., 2010), was diluted in
223 distilled water and given via gavage at a dosage of 100 mg/kg/day (da Silva-Maia et al., 2019).
224 Mesalamine was administrated for seven days, from the day of the induction until the end of the
225 experiment.

226 The dietary intake (g) and body weight (g) were measured during the whole experiment.
227 Additionally, for the period of colitis, the Disease Activity Index (DAI) was assessed (days 14, 15,
228 17, 19, and 20), according to described by Gommeaux et al. (2007). The DAI evaluates the body
229 weight loss, anal bleeding, and stool consistency (Gommeaux et al., 2007). Photographs of the rat's
230 stool and anus were taken to analyze anal bleeding and stool consistency. A blinded researcher to

231 animals' identification and with experience in IBDS, did the evaluations. A photographic
232 representation of the scores of bleeding and stool consistency from this study was created similarly
233 to Nascimento, Lima, et al. (2020) to showcase the differences between the score's degrees. A
234 Nikon® SLR D3100 digital camera with 14.2-megapixel resolution and an 18-55 mm lens was
235 utilized.

236

237 **2.3.3. Euthanasia and tissue measurements**

238 Euthanasia was performed on day 21 by exsanguination through cardiac puncture.
239 Intraperitoneal administration of ketamine (300 mg/kg of body weight) and xylazine (30 mg/kg of
240 body weight) were used to anesthetize the animals.

241 Colon was dissected, cleaned with saline solution, weighted (g), and measured with a ruler
242 (cm). The colon weight/length (g/cm) ratio was analyzed. Brownish necrotic lesions are common
243 features of TNBS-induced colitis (De Almeida et al., 2015; Paiotti et al., 2013); therefore, their
244 width (cm), length (cm), and estimated area (width x length, cm²) were measured for this study.
245 Additionally, the relation between the larger necrotic lesion length (cm) and the colon length (cm)
246 was evaluated as an indicator of the colon proportion (%) affected by the disease. A small portion of
247 the distal colon was separated for histology. The remaining, composed of proximal, middle, and
248 distal colon, and rectum, were mixed and preserved at -80°C for further analyses (inflammation and
249 oxidative stress).

250 Blood was centrifuged, and the serum was collected and stored at -80°C for posterior
251 antioxidant analyses. Feces from the cecum and colon were collected and kept at -80°C for the
252 analysis of the concentration of SCFAs. The liver, spleen, and kidney were weighted (g). The
253 carcasses of the animals and unused tissues or organs were discarded as biological materials.

254

255 **2.4. Histopathology**

256 A sample of the distal colon was disposed in a small piece of paper and preserved in
257 formaldehyde 4% (v/v) until histological processing. Briefly, samples were dehydrated with
258 increasing ethanol concentrations, included in paraffin blocks, and subjected to sectioning (4 μm)
259 follow by insertion in slides. Slides were standardly stained with hematoxylin and eosin and
260 analyzed by a specialist, blind to the identification of the groups, according to a score modified
261 from Dieleman et al. (1998) (**Table 1**). Photos were taken at 10x/0.25 magnification, 6.31x/0.25
262 AxioCam ICc5 (Zeiss, Germany).

263

264 **2.5. Antioxidant capacity of the serum**

265 The total antioxidant capacity of the serum was evaluated by two methods, the ferric-
266 reducing antioxidant power (FRAP) and the oxygen-radical absorbing capacity (ORAC). Trolox
267 was utilized as a standard for both analyses. Before the tests, samples were submitted to treatment
268 with metaphosphoric acid in order to precipitate proteins and extract the antioxidant substances
269 (Leite et al., 2011).

270 For the FRAP analysis, samples were added with a solution composed of 2,4,6-tripyridyl-S-
271 triazine, ferric chloride, and acetate buffer at proportions of 1:1:10, respectively. Samples were
272 included in a microplate and incubated for 30 min at 37°C. The absorbance was read at 595 nm
273 using an Epoch™ spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA), which was
274 utilized for all the applicable analyses. The results were expressed in μmol of Trolox equivalent/mL
275 of serum (Benzie & Strain, 1996).

276 For the ORAC analysis, in a microplate, samples were added with fluorescein and incubated
277 for 10 min at 37°C. After that, 2,2'-azobis(2-amidinopropane) dihydrochloride was added, and the
278 fluorescence was read for 80 min with filters set at 520 nm for emission and 485 nm for excitation.
279 The area under the curve was calculated, and the results were expressed in μmol of Trolox
280 equivalent/mL of serum (Ou et al., 2013).

281

282 **2.6. Inflammation and oxidative stress analyses**

283 Colonic samples were homogenized in a potassium phosphate buffer (75 mM, pH 7.4) and
284 centrifuged at 10,000 rpm for 30 min. The supernatant was collected and subjected to protein
285 quantification (mg of bovine serum albumin/mL) by the Bradford assay (Bradford, 1976).
286 Supernatants were utilized for the determination of MPO and cytokines concentration, and the
287 oxidative stress analyses.

288 The concentration of MPO (U/g protein), tumor necrosis factor-alpha (TNF- α), and
289 interleukin-1beta (IL-1 β) (ng/g of protein or mL of serum) were determined as indicators of
290 inflammation. MPO was determined by mixing the diluted homogenates (1:4) or a standard with a
291 cocktail of O-dianisidine dihydrochloride and hydrogen peroxide. MPO was utilized as the
292 standard. Absorbance was read every min for 10 min at 460 nm, and the area under the curve was
293 calculated (Winterbourn et al., 1975). The concentrations of the proinflammatory cytokines TNF- α
294 and IL-1 β in colon homogenates and serum were determined by ELISA, following the protocols of
295 commercial kits.

296 The activity or concentration of CAT (U/g of protein), glutathione reductase (GR)
297 (consumed NADPH/min/g of protein), GSH (nmol of GSH/mg of protein), malondialdehyde
298 (MDA) (nmol of MDA/g of protein), and superoxide dismutase (SOD) (SOD/g of protein) were
299 determined as indicators more closely related to oxidative stress.

300 The CAT assay was realized by mixing first the diluted homogenates (1:2) with hydrogen
301 peroxide and incubating for two min at 37°C. After that, ammonium metavanadate was included,
302 and the absorbance was read after 10 min at 452 nm. CAT was utilized as a standard. CAT activity
303 was determined by a logarithmic formula, adapted from a recent methodological approach (Hadwan
304 & Ali, 2018).

305 For the GR analysis, homogenates (diluted at 1:1 or not diluted, depending on the sample)
306 were added with a cocktail containing reduced nicotinamide-adenine dinucleotide phosphate,

307 oxidized glutathione, and ethylenediaminetetraacetic acid. The reading was realized at 340 nm per
308 min for 10 min and the area under the curve was calculated (Carlberg & Mannervik, 1985).

309 GSH was determined by first mixing a tris reagent plus ethylenediaminetetraacetic acid
310 buffer with diluted homogenates (1:4) or a standard and read at 421 nm. This step was followed by
311 the inclusion of 5,5'-dithiobis-(2-nitrobenzoic acid) and a 15 min incubation period. The second
312 reading was also done at 421 nm. GSH was utilized as the standard. The calculation was realized by
313 subtracting the first reading data from the second one, followed by linear regression (Yoshikawa et
314 al., 1993).

315 The concentration of MDA was determined by the thiobarbituric acid reactive substances
316 (TBARS) method. Briefly, samples or a standard were mixed with sodium dodecyl sulfate and a
317 solution containing thiobarbituric acid, sodium hydroxide, and acetic acid. This mixture was left for
318 60 min in a boiling water bath and then cooled for 10 min. Samples were centrifuged at 10,000 rpm
319 for 10 min at low temperature (4°C). The absorbance of the supernatant was read at 532 nm. A
320 standard curve was made using 1,1,3,3-tetraethoxypropane, and the results were expressed as nmol
321 MDA/g protein (Ohkawa et al., 1979).

322 SOD was quantified by mixing the diluted homogenates (1:4) with a cocktail composed of
323 hypoxanthine, xanthine oxidase, and nitro blue tetrazolium chloride. Absorbance was read every
324 min for 10 min at 560 nm, and the area under the curve was calculated (Winterbourn et al., 1975).

325

326 **2.7. Short-chain fatty acids concentration**

327 Feces sample preparation followed the protocol described by Zhao et al. (2006), with
328 modifications. Approximately 300 mg of stool was diluted in distilled water (1:6) and mixed with
329 HCl for adjustment in pH 2. Afterward, the samples were centrifuged at 3000 rpm for 60 min. The
330 supernatant was collected and mixed with the internal standard 2-ethyl-butyric acid. Once prepared,
331 the samples were injected into a gas chromatograph coupled to a flame ionization detector. The
332 samples were injected with an auto-injector brand Shimadzu, model Ai20, in a GC-FID Shimadzu,

333 model GC-2010 plus, equipped with a capillary column of fused silica NUKOI (30 m x 0.25 mm,
334 inside diameter x 0.25 μ m). The chromatographic conditions were as follow: injector at 200°C
335 operating in split mode 1:5 for 1.0 min; helium carrier gas at 1 mL/min; oven temperature ramp
336 starting at 100°C, with an increase of 8°C/min to 190°C, remaining at this temperature for 3.25 min;
337 and detector at 200°C. The co-injection of authentic standards identified the analytes. All
338 chromatographic analyzes were performed in triplicate (Zhao et al., 2006).

339

340 **2.8. Statistical analysis and Pearson's correlation**

341 Results are presented as mean \pm standard deviation (SD) for data from the chemical
342 characterization and as mean \pm standard error of the mean (SEM) for data from the biological
343 analyses.

344 For data from the biological analysis, first, outliers were discarded after realizing the Grubbs
345 test (5%). Then, One-way ANOVA followed by Tukey (parametric data) was utilized when making
346 comparisons among all experimental groups. Two-way ANOVA followed by Tukey (parametric
347 data) was utilized for analyses over time (body weight, DAI). Data between groups were considered
348 statistically different when $p < 0.05$.

349 Data were submitted to Pearson's correlation and included in a correlation matrix. The
350 correlations were only considered parameters previously attested to be significantly altered by the
351 induction of colitis or the intervention with the HT ($p < 0.05$). Groups without colitis induction (C,
352 H) were not included, as they could bias the results. Pearson's r placed between 0.70 and 1
353 (positive) or -0.70 and -1 (negative) were classified as highly correlated (Hinkle et al., 2003). A
354 heatmap was created to represent the correlation matrix.

355 Analyses were performed using GraphPad Prism.

356

357 **3. RESULTS AND DISCUSSION**

358 **3.1. Tea characterization and dosage of compounds administrated to rats**

359 The extraction yield was about 20% in mass, meaning that 200 mg of HT was obtained from
360 1 g of the dry ground plant. The contents of TPC, TFC and TPOC obtained from HT were $165.55 \pm$
361 2.20 mg of gallic acid equivalent/g of dry tea, 18.8 ± 0.70 mg of quercetin equivalent/g of dry tea,
362 and 15.35 ± 0.60 mg of cyanidin equivalent/g of dry tea, respectively. The levels found from the
363 three analyses are in accordance with recent studies using similar plant species and portions (Issaadi
364 et al., 2020; Ngoc et al., 2019). By utilizing only water for the extraction of the ground plant, the
365 results of TPC (33.11 mg/g of dry plant) shown in the present study are comparable or even higher
366 than less green extracts utilized by the literature (Edwards et al., 2012). For example, in a study by
367 Alirezalu et al. (2018), the TPC of different *Crataegus* species (flowers and leaves), extracted with
368 methanol/water (80%, v/v), ranged between 20 to 50 mg/g. Also, in a study by Keser et al. (2014),
369 the polyphenol content of the aqueous extract of *Crataegus monogyna* leaves and flowers was
370 found to be higher than the extract with 100% ethanol, while also possessing a high antioxidant
371 activity (Keser et al., 2014). Such findings suggest that the use of water for the extraction of
372 hawthorn can be equally important to other solvents, as far as the dry plant is ground (< 1 mm).

373 The main compounds found in the HT were flavonoids, like chlorogenic acid, vitexin 2-O-
374 rhamnoside, pinnatifinoside A, hyperoside, and apigenin-C-hexoside, as detected by the UHPLC
375 retention time (**Table 2**) and peak (**Fig. 1A**) profiles. Especially, vitexin 2-O-rhamnoside presented
376 the highest relative peak area (**Fig. 1B**), as later quantified. The content of this flavonoid by
377 UHPLC was determined to be 3.53 ± 0.036 mg/g of dry plant or 17.66 ± 0.18 mg/g of dry HT.
378 Vitexin 2-O-rhamnoside is the main flavonoid found in hawthorn species, with content reaching up
379 to 6.6 mg/g of dry plant (Martino et al., 2008) or 26 mg/g of the tea (i.e., 5.2 mg/g of dry tea) (Ngoc
380 et al., 2019). Studies have shown the capacity of vitexin 2-O-rhamnoside against induced-oxidative
381 stress damage (Wei et al., 2014) and endothelial injury (Zhu et al., 2006), making it also a probable
382 candidate for inflammatory intestinal conditions. However, such flavonoid has poor oral
383 bioavailability in rats (less than 5%) (Gao et al., 2016), which could indicate its biotransformation

384 by the microbiota in similarity to other prebiotic-like flavonoids (e.g., anthocyanins) (Kawabata et
385 al., 2019), but studies are needed to investigate such assumption.

386 Considering the results from the chemical characterization of the hawthorn plant, rats
387 received 16.5 mg of total phenolic compounds/kg of body weight and 1.76 mg of vitexin 2-O-
388 rhamnoside/kg of body weight daily during 14 days for prevention and the following 7 days while
389 with colitis.

390

391 3.2. Macroscopic and microscopic parameters

392 Colitis was successfully induced in rats, as seen by an increased body weight loss (**Fig. 2A**)
393 and high DAI after one day from the colitis induction ($p < 0.01$) (**Fig. 2B**). In general, animals with
394 colitis presented softening of the stools and slight bleeding, as shown by the photographic
395 representation of their respective scores in **Figs. 3** and **4**, respectively. As expected, the treatment
396 with 100 mg/kg of mesalamine recovered the animals' body weight at the end of the experiment
397 ($p < 0.001$) compared to the *CC* group. Mesalamine is commonly utilized for IBDs, as it can block
398 cyclooxygenases, interleukins, and TNF- α , therefore controlling the inflammatory process and
399 contributing to preventing common intestinal symptoms and body weight loss (Lacucci et al.,
400 2010). HT could not significantly reverse the body weight loss ($p = 0.0560$) in rats with colitis
401 (group *HC*), but animals tended to return to their initial body weight before colitis induction (**Fig.**
402 **2A**).

403 Only the rats in the *CC* group presented a high colon weight/length ratio ($p < 0.05$) compared
404 to the *C* group (**Fig. 2C**), potentially indicating the anti-inflammatory effects of the interventions
405 with mesalamine and HT. The occurrence of necrotic lesions was evident in all rats that received
406 TNBS (**Fig. 2D**). Both mesalamine and HT significantly decreased the length ($p < 0.001$) and the
407 area ($p < 0.01$) of the most extensive brownish necrotic lesion, as well as the proportion of the colon
408 affected by TNBS ($p < 0,05$) compared to the *CC* group. No differences were found between the *MC*
409 and *HC* groups regarding such parameters (**Table 3**).

410 The anti-necrotic potential of extracts from hawthorn is still poorly documented (Fujisawa et
411 al., 2005; Zapatero, 1999), but evidence indicates that flavonoids and phenolic acids (e.g.,
412 chlorogenic acid), which were identified in the HT of our study (**Fig. 1, Table 2**), possess such
413 properties. For example, chlorogenic acid, a polyphenol found in coffee beans, apple, and hawthorn
414 species (Alirezalu et al., 2018), and the vitexin-rich plant *Tragopogon graminifolius*, are effective in
415 reducing the typical macroscopic and necrotic lesions of TNBS-induced colitis (Farzaei et al., 2015;
416 Zatorski et al., 2015; Zhou et al., 2016). Although necrosis is not a typical documented finding of
417 patients with IBDs, it can work as a parameter to measure the possible anti-inflammatory and pro-
418 homeostatic effects of natural products.

419 Although alterations in the liver, spleen, and kidney are described in few studies that use the
420 model of TNBS-induced colitis (Jang et al., 2018; Patel & Trivedi, 2017; Zhi et al., 2017), in the
421 present study, no differences were found between the experimental groups regarding the
422 macroscopic aspect and weight of such organs (results not shown).

423 As far as the histological analysis, TNBS promoted extensive and severe ulceration in the
424 epithelium, with loss of crypts, and a high inflammatory income in the mucosa and submucosa (**Fig.**
425 **5**). Results from the histological grading significantly shown this for all colitis groups ($p < 0.05$).
426 Mesalamine ($p = 0.103$) and HT ($p = 0.318$) did not statistically decrease the histological score (**Table**
427 **3**), which could probably be related to two possibilities: 1. as expected for the TNBS model
428 (Antoniou et al., 2016), the microscopic severity was extremely high, especially in the area selected
429 for histology (distal colon), making it difficult for a clear observation of the benefits of the
430 treatments; 2. the HT only had a mild preventive effect that could not be translated in the
431 microscopic analysis. The next step would be to perform a study with other colitis model to
432 understand if HT could exert preventive effects in the histology of the damaged intestinal mucosa.
433 Cinthia B.B. Cazarin et al. (2014), for example, by using the *Passiflora edulis* peel (rich in
434 polyphenols and dietary fibers) could not find statistical significance in the microscopic score of
435 rats with TNBS-induced colitis, but when they utilized the dextran sodium sulfated (DSS) model, a

436 significant reduction in the histological damage in the colon was observed (Cintia Bau Beum
437 Cazarin et al., 2016). The DSS model results in less severe inflammation and more superficial
438 ulcers in rats when compared to the TNBS acute model (Catana et al., 2018), which typically
439 triggers extensive transmural inflammation (Antoniou et al., 2016). According to a recent review
440 (Nascimento, Machado, et al., 2020), the DSS model best mimics the inflammation of UC, while
441 the TNBS model, the inflammation of CD. In studies with colitis using products from hawthorn
442 berries, the histological damage is improved, however, the models associated with the results are
443 acetic acid and DSS, and not TNBS (Fujisawa et al., 2005; Guo et al., 2021; Malekinejad et al.,
444 2013).

445

446 **3.3. Serum antioxidant capacity**

447 No statistical differences were found between all groups regarding the results from the
448 FRAP and ORAC methods, meaning that the TNBS model did not worsen the systemic antioxidant
449 capacity; neither did the intervention with HT increase it (**Table 4**). In a study by Luana H. Maurer
450 et al. (2020), similarly, ORAC and FRAP data were not changed by the TNBS-colitis model or by
451 the intervention with a rich source of polyphenols (Luana H. Maurer et al., 2020). Since our study
452 utilized an acute colitis model, we understand that the serum was not deeply affected. Maybe, a
453 different dosage of HT may be necessary to achieve significance for such analyses.

454

455 **3.4. Concentration or activity of inflammatory proteins and oxidative metabolism enzymes**

456 As expected, MPO and IL-1 β levels were significantly increased by the inflammatory
457 process in the colon ($p < 0.01$). Both mesalamine and HT decreased the concentrations of MPO and
458 IL-1 β ($p < 0.05$) (**Table 4**). Similarly, Liu et al. (2020) and Malekinejad et al. (2013), by using
459 flavonoid-rich extracts from hawthorn berries, also found reduced levels of IL-1 β and MPO,
460 respectively, in their pre-clinical studies with colitis. Concentrations of IL-1 β in serum and TNF- α
461 in colon and serum were not changed in our study (**Table 4**).

462 Besides the common pathway of release of the IL-1 β by macrophages (caspase-1 activation
463 by inflammasome), neutrophil proteases can also cleave the precursor of this cytokine into its
464 biologically active forms (Guma et al., 2009), following tissue injury and cell necrosis. Also, to help
465 create an IL-1 β -dependent inflammatory response (Lopez-Castejon & Brough, 2011), neutrophils
466 can express MPO, a local mediator of tissue damage and inflammation ignition (Aratani, 2018).
467 Patients with IBDs possess high serum and fecal levels of IL-1 β and MPO (Peterson et al., 2007;
468 Vasilyeva et al., 2016), and the typical inflammation of IBDs is largely attributed to the
469 accumulation (UC) or impaired function (CD) of neutrophils (Wéra et al., 2016). In the present
470 study, HT was able to decrease the levels of IL-1 β and MPO in the colon, indicating its potential to
471 block or regulate intestinal inflammations associated with the accumulation and activation of
472 intestinal mucosal neutrophils (Therrien et al., 2019).

473 Regarding the results of oxidative metabolism enzymes, CAT activity was increased by the
474 colitis model ($p < 0.01$). Experimental studies with mice supplemented with strains of genetically
475 modified bacteria found that elevated CAT activity in a TNBS-induced CD murine model is linked
476 to a decreased likelihood of worsening the disease and developing colon cancer (LeBlanc et al.,
477 2011). Observing **Table 4**, only the colitis group treated with the HT (group *HC*) showed a
478 normalization in CAT levels ($p < 0.01$), demonstrating a superior effect of a natural alternative
479 intervention in comparison with a drug. Additionally, the colitis group receiving HT highly
480 increased the levels of GR in comparison to *CC* and *MC* ($p < 0.001$) (**Table 4**). This increase
481 possibly happened in response to the production of glutathione peroxidase (GPx), an antioxidant
482 enzyme that works in conjunction with CAT by transforming hydrogen peroxide (H₂O₂) into water
483 (H₂O), thus decreasing oxidative stress caused by inflammation (Ighodaro & Akinloye, 2018).
484 Briefly, in order for the peroxisome organelle to obtain GPx, it is necessary to convert reduced
485 glutathione (GSH) into its oxidized form (GSSH). For the regeneration of GSH (glutathione cycle),
486 the presence of GR is necessary (Moura et al., 2015).

487 Both CAT and GR are also known for their role in lipid peroxidation. CAT acts as a first-
488 line defense against the formation of free radicals, while GR is responsible for the regeneration of
489 GHS, the body master's antioxidant (Fagan & Palfey, 2010; Ighodaro & Akinloye, 2018; Pérez et
490 al., 2017). According to recent studies, GR can be increased in rats with improved TNBS-induced
491 colitis, and that received rich sources of flavonoids (jaboticaba peel and grape peel residues) (da
492 Silva-Maia et al., 2019; Luana Haselein Maurer et al., 2019). This scenario suggests the importance
493 of GR restoration to ameliorate intestinal conditions. On the other side, despite the well-known
494 regulating role of CAT, in the present study, its activity was increased after colitis induction. This
495 modification is probably due to adaptive or defective mechanisms, as this enzyme has also been
496 found highly expressed in neutrophils from the intestinal mucosa of patients with IBDs (Kruidenier
497 et al., 2003). Therefore, besides acting in the MPO-IL-1 β axis, HT could also positively interfere in
498 the mechanics of CAT and GR, first by avoiding the excessive or defective action of CAT, then by
499 highly increasing the levels of GR to control the propagation of reactive oxygen species and restore
500 redox balance.

501 The levels of SOD, TBARS, and GSH in the colon were not changed either by the colitis
502 model or the interventions (**Table 4**).

503

504 **3.5. Concentration of short-chain fatty acids**

505 The extract of the *Crataegus oxyacantha* leaves is reported to increase the growth of
506 *Bifidobacterium* and *Lactobacillus* species and the concentration of acetic acid, according to an *in*
507 *vitro* study using skim milk as a fermentable matrix (Khaleel & Haddadin, 2013). Another study
508 showed that flavonoids, including chlorogenic acid, which is abundant in *Crataegus oxyacantha*,
509 slightly increased the counts of *Bifidobacterium spp.* and the concentration of SCFAs *in vitro*
510 (Parkar et al., 2013). However, in the present study, the concentration of SCFAs (total, acetic,
511 butyric, propionic) in feces from both cecum and colon of rats was not altered by the HT.

512 Additionally, these compounds were not modulated by the TNBS-colitis induction model (data not

513 snown). Perhaps, the polyphenols dosage in this study may not be sufficient to increase the levels of
514 SCFAs, or the treatment period was short. Since our study used oral gavage to inoculate the
515 proposed treatment, we decided to not extend its duration. Gavage, especially if done for a
516 prolonged period and repeatedly, may cause reflux, irritation, and inflammation of the esophagus
517 (Damsch et al., 2011; Kinder et al., 2014). Longer treatment periods using diets enriched with
518 hawthorn products may encounter better results as far as SCFAs levels.

519 A recent investigation using a treatment with hawthorn for chemically-induced colitis found
520 microbiota modulation and increased levels of SCFAs *in vivo*. However, a diversity of aspects were
521 different from our study, which include: the animals used (C57BL/6J mice), the colitis model
522 (DSS), the fraction and species of the hawthorn plant (fruit of *Crataegus pinnatifida*), and
523 especially, the treatment applied and its period (an extracted polysaccharide for 42 days) (Guo et al.,
524 2021). Such promising evidence indicates that the microbiome-related aspects of the therapy with
525 the hawthorn plant are worthy of investigation and need further experiments to confirm its potential
526 *in vivo*.

527

528 3.6. Correlation matrix

529 Colon weight/length ratio, the area of the necrotic lesions, and IL-1 β levels presented the
530 most numerous and diverse correlations. On the contrary, the DAI at its peak day had overall weak
531 correlations with all other parameters (**Fig. 6**).

532 The colon weight/length ratio, which is regarded as an inflammatory index (Sánchez-
533 Fidalgo et al., 2010), was highly correlated with almost all the lesion parameters and the levels of
534 IL-1 β . On the other side, this cytokine was positively correlated with MPO levels ($r=0.846$),
535 probably due to mechanisms previously mentioned, associated with neutrophil infiltration and the
536 release of inflammatory markers in the colonic mucosa. MPO also showed a high negative
537 correlation with body weight alteration ($r=-0.708$). In a trial with patients with UC, MPO and IL-1 β
538 had good correlations with the histological, endoscopic, and clinical severity of the disease

539 (Peterson et al., 2007). This scenario suggests the possibility of measuring such markers to improve
540 prognostic in IBDs, in addition to targeting compounds capable of specifically blocking them, such
541 as the ones found in the HT.

542 There was also a negative correlation ($r=-0.732$) between the levels of CAT and GR. As
543 previously mentioned, this data shows that HT is mechanistically acting through an oxidative stress
544 pathway, mainly by affecting in opposite ways GR and CAT in order to prevent the spread of free
545 radicals and consequently oxidative stress.

546 Although the interventions did not reduce the histological score, there was a high positive
547 correlation of such parameter with the necrotic lesion area ($r=0.708$), which was diminished by the
548 HT.

549

550 4. CONCLUSIONS

551 Animal studies directed towards IBDs lack analysis or do not present an adequate
552 experimental model (e.g., acetic acid-induced colitis). This study is, to the best of our knowledge,
553 the first successful investigation of the effects of *Crataegus oxyacantha* in IBDs. A HT prepared
554 from the flowering tops (leaves and flowers), a source of polyphenols, effectively decreases
555 inflammatory mediators (IL-1 β , MPO) and regulates oxidative stress in TNBS-induced colitis.
556 Additionally, administration of HT via oral gavage does not cause any harm in healthy rats. Such
557 findings indicate that the hawthorn plant may serve as an alternative or complementary natural
558 remedy that extends beyond its well-known cardiovascular protective and anti-hypertensive roles.

559

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568

569 **DECLARATION OF INTEREST**

570 None.

571

572 **REFERENCES**

573 Alirezalu, A., Salehi, P., Ahmadi, N., Sonboli, A., Aceto, S., Maleki, H. H., & Ayyari, M. (2018).

574 Flavonoids profile and antioxidant activity in flowers and leaves of hawthorn species

575 (*Crataegus* spp.) from different regions of Iran. *International Journal of Food Properties*.

576 <https://doi.org/10.1080/10942912.2018.1446146>

577 Antoniou, E., Margonis, G. A., Angelou, A., Pikouli, A., Argiri, P., Karavokyros, I., Papalois, A., &

578 Pikoulis, E. (2016). The TNBS-induced colitis animal model: An overview. In *Annals of*

579 *Medicine and Surgery*. <https://doi.org/10.1016/j.amsu.2016.07.019>

580 Aratani, Y. (2018). Myeloperoxidase: Its role for host defense, inflammation, and neutrophil

581 function. In *Archives of Biochemistry and Biophysics*.

582 <https://doi.org/10.1016/j.abb.2018.01.004>

583 Benzie, I. F. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of

584 "antioxidant power": The FRAP assay. *Analytical Biochemistry*.

585 <https://doi.org/10.1006/abio.1996.0292>

586 Billing, E., Bech-Andersen, J., & Lelliott, R. A. (1974). Fireblight in Hawthorn in England and

587 Denmark. *Plant Pathology*. <https://doi.org/10.1111/j.1365-3059.1974.tb01837.x>

588 Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities

589 of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*.

590 [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)

- 591 Campos, M. K. S. (2019). *Bioactive Compounds: Health Benefits and Potential Applications*.
592 Elsevier.
- 593 Cardona, F., Andrés-Lacueva, C., Tulipani, S., Tinahones, F. J., & Queipo-Ortuño, M. I. (2013).
594 Benefits of polyphenols on gut microbiota and implications in human health. In *Journal of*
595 *Nutritional Biochemistry*. <https://doi.org/10.1016/j.jnutbio.2013.05.001>
- 596 Carlberg, I., & Mannervik, B. (1985). [59] Glutathione reductase. *Methods in Enzymology*.
597 [https://doi.org/10.1016/S0076-6879\(85\)13062-4](https://doi.org/10.1016/S0076-6879(85)13062-4)
- 598 Catana, C. S., Magdas, C., Tabaran, F. A., Craciun, E. C., Deak, G., Magdas, V. A., Cozma, V.,
599 Gherman, C. M., Berindan-Neagoe, I., & Dumitrascu, D. L. (2018). Comparison of two
600 models of inflammatory bowel disease in rats. *Advances in Clinical and Experimental*
601 *Medicine*. <https://doi.org/10.17219/acem/69134>
- 602 Cazarin, Cinthia B.B., da Silva, J. K., Colomeu, T. C., Batista, Â. G., Vilella, C. A., Ferreira, A. L.,
603 Junior, S. B., Fukuda, K., Augusto, F., de Meirelles, L. R., Zollner, R. de L., & Junior, M. R.
604 M. (2014). Passiflora edulis peel intake and ulcerative colitis: Approaches for prevention and
605 treatment. *Experimental Biology and Medicine*. <https://doi.org/10.1177/1535370214525306>
- 606 Cazarin, Cinthia Baú Betim, Rodriguez-Nogales, A., Algieri, F., Utrilla, M. P., Rodríguez-Cabezas,
607 M. E., Garrido-Mesa, J., Guerra-Hernández, E., Braga, P. A. de C., Reyes, F. G. R., Maróstica,
608 M. R., & Gálvez, J. (2016). Intestinal anti-inflammatory effects of Passiflora edulis peel in the
609 dextran sodium sulphate model of mouse colitis. *Journal of Functional Foods*.
610 <https://doi.org/10.1016/j.jff.2016.08.020>
- 611 Chang, Q., Zuo, Z., Harrison, F., & Chow, M. S. S. (2002). Hawthorn. In *Journal of Clinical*
612 *Pharmacology*. <https://doi.org/10.1177/00970002042006003>
- 613 da Silva-Maia, J. K., Batista, Â. G., Cazarin, C. B. B., Soares, E. S., Junior, S. B., Leal, R. F., da
614 Cruz-Höfling, M. A., & Maróstica Junior, M. R. (2019). Aqueous extract of Brazilian berry
615 (Myrciaria jaborcaba) peel improves inflammatory parameters and modulates Lactobacillus
616 and Bifidobacterium in rats with induced-colitis. *Nutrients*.

- 617 <https://doi.org/10.3390/nu11112770>
- 618 Dahmer, S., & Scott, E. (2010). Health effects of hawthorn. In *American Family Physician*.
- 619 Dalli, E., Colomer, E., Tormos, M. C., Cosín-Sales, J., Milara, J., Esteban, E., & Sáez, G. (2011).
620 *Crataegus laevigata* decreases neutrophil elastase and has hypolipidemic effect: A randomized,
621 double-blind, placebo-controlled trial. *Phytomedicine*.
622 <https://doi.org/10.1016/j.phymed.2010.11.011>
- 623 Damsch, S., Eichenbaum, G., Tonelli, A., Lammens, L., Van den Bulck, K., Feyen, B.,
624 Vandenberghe, J., Megens, A., Knight, E., & Kelley, M. (2011). Gavage-related reflux in rats:
625 identification, pathogenesis, and toxicological implications (review). *Toxicologic Pathology*,
626 39(2), 348–360. <https://doi.org/10.1177/0192623310388431>
- 627 De Almeida, C. S., Andrade-Oliveira, V., Câmara, N. O. S., Jacysyn, J. F., & Faquim-Mauro, E. L.
628 (2015). Crotoxin from *Crotalus durissus terrificus* is able to down-modulate the acute intestinal
629 inflammation in mice. *PLoS ONE*. <https://doi.org/10.1371/journal.pone.0121427>
- 630 Dieleman, L. A., Palmen, M. J. H. J., Akol, H., Bloemena, E., Peña, A. S., Meuwissen, S. G. M., &
631 Van Rees, E. P. (1998). Chronic experimental colitis induced by dextran sulphate sodium
632 (DSS) is characterized by Th1 and Th2 cytokines. *Clinical and Experimental Immunology*.
633 <https://doi.org/10.1046/j.1365-2249.1998.00728.x>
- 634 Edwards, J. E., Brown, P. N., Talent, N., Dickinson, T. A., & Shipley, P. R. (2012). A review of the
635 chemistry of the genus *Crataegus*. In *Phytochemistry*.
636 <https://doi.org/10.1016/j.phytochem.2012.04.006>
- 637 Elango, C., & Devaraj, S. N. (2010). Immunomodulatory effect of Hawthorn extract in an
638 experimental stroke model. *Journal of Neuroinflammation*. [https://doi.org/10.1186/1742-2094-](https://doi.org/10.1186/1742-2094-7-97)
639 7-97
- 640 Elango, C., Jayachandaran, K. S., & Niranjali Devaraj, S. (2009). Hawthorn extract reduces infarct
641 volume and improves neurological score by reducing oxidative stress in rat brain following
642 middle cerebral artery occlusion. *International Journal of Developmental Neuroscience*.

- 643 <https://doi.org/10.1016/j.ijdevneu.2009.08.008>
- 644 Fagan, R. L., & Palfey, B. A. (2010). Flavin-dependent enzymes. In *Comprehensive Natural*
645 *Products II: Chemistry and Biology*. <https://doi.org/10.1016/b978-008045382-8.00135-0>
- 646 Farzaei, M. H., Ghasemi-Niri, S. F., Abdolghafari, A. H., Baeri, M., Khanavi, M., Navaei-Nigjeh,
647 M., Abdollahi, M., & Rahimi, R. (2015). Biochemical and histopathological evidence on the
648 beneficial effects of *Tragopogon graminifolius* in TNBS-induced colitis. *Pharmaceutical*
649 *Biology*. <https://doi.org/10.3109/13880209.2014.923004>
- 650 Fujisawa, M., Oguchi, K., Yamaura, T., Suzuki, M., & Cyong, J. C. (2005). Protective effect of
651 hawthorn fruit on murine experimental colitis. *American Journal of Chinese Medicine*.
652 <https://doi.org/10.1142/S0192415X05002849>
- 653 Gao, Y., Du, Y., Ying, Z., Leng, A., Zhang, W., Meng, Y., Li, C., Xu, L., Ying, X., & Kang, T.
654 (2016). Hepatic, gastric and intestinal first-pass effects of vitexin-2''-O-rhamnoside in rats by
655 ultra-high-performance liquid chromatography. *Biomedical Chromatography*.
656 <https://doi.org/10.1002/bmc.3522>
- 657 Gommeaux, J., Cano, C., Garcia, S., Gironella, M., Pietri, S., Culcasi, M., Pébusque, M.-J.,
658 Malissen, B., Dusetti, N., Iovanna, J., & Carrier, A. (2007). Colitis and Colitis-Associated
659 Cancer Are Exacerbated in Mice Deficient for Tumor Protein 53-Induced Nuclear Protein 1.
660 *Molecular and Cellular Biology*, 27(6), 2215–2228. <https://doi.org/10.1128/MCB.01454-06>
- 661 Guma, M., Ronacher, L., Liu-Bryan, R., Takai, S., Karin, M., & Corr, M. (2009). Caspase 1-
662 independent activation of interleukin-1 β in neutrophil-predominant inflammation. *Arthritis*
663 *and Rheumatism*. <https://doi.org/10.1002/art.24959>
- 664 Guo, C., Wang, Y., Zhang, S., Zhang, X., Du, Z., Li, M., & Ding, K. (2021). *Crataegus pinnatifida*
665 polysaccharide alleviates colitis via modulation of gut microbiota and SCFAs metabolism.
666 *International Journal of Biological Macromolecules*, 181, 357–368.
667 <https://doi.org/10.1016/J.IJBIOMAC.2021.03.137>
- 668 Hadwan, M. H., & Ali, S. kadhum. (2018). New spectrophotometric assay for assessments of

- 669 catalase activity in biological samples. *Analytical Biochemistry*.
670 <https://doi.org/10.1016/j.ab.2017.11.013>
- 671 Hatipoğlu, M., Saflam, M., Köseoğlu, S., Köksal, E., Keleş, A., & Esen, H. H. (2015). The
672 effectiveness of *Crataegus orientalis* M Bieber. (Hawthorn) extract administration in
673 preventing alveolar bone loss in rats with experimental periodontitis. *PLoS ONE*.
674 <https://doi.org/10.1371/journal.pone.0128134>
- 675 Hinkle, D. E., Wiersma, W., & Jurs, S. G. (2003). *Applied statistics for the behavioral sciences*
676 (Mass: Houghton Mifflin (ed.); 3rd ed.).
- 677 Horne, R., Parham, R., Driscoll, R., & Robinson, A. (2009). Patient's attitudes to medicines and
678 adherence to maintenance treatment in inflammatory bowel disease. *Inflammatory Bowel*
679 *Diseases*. <https://doi.org/10.1002/ibd.20846>
- 680 Ighodaro, O. M., & Akinloye, O. A. (2018). First line defence antioxidants-superoxide dismutase
681 (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire
682 antioxidant defence grid. *Alexandria Journal of Medicine*.
683 <https://doi.org/10.1016/j.ajme.2017.09.001>
- 684 Issaadi, O., Fibiani, M., Picchi, V., Scalzo, R. Lo, & Madani, K. (2020). Phenolic composition and
685 antioxidant capacity of hawthorn (*Crataegus oxyacantha* L.) flowers and fruits grown in
686 Algeria. *Journal of Complementary and Integrative Medicine*. [https://doi.org/10.1515/jcim-](https://doi.org/10.1515/jcim-2018-0125)
687 [2018-0125](https://doi.org/10.1515/jcim-2018-0125)
- 688 Jang, S. E., Jeong, J. J., Kim, J. K., Han, M. J., & Kim, D. H. (2018). Simultaneous Amelioration
689 of Colitis and Liver Injury in Mice by *Bifidobacterium longum* LC67 and *Lactobacillus*
690 *plantarum* LC27. *Scientific Reports*. <https://doi.org/10.1038/s41598-018-25775-0>
- 691 Kawabata, K., Yoshioka, Y., & Terao, J. (2019). Role of intestinal microbiota in the bioavailability
692 and physiological functions of dietary polyphenols. In *Molecules*.
693 <https://doi.org/10.3390/molecules24020370>
- 694 Keser, S., Celik, S., Turkoglu, S., Yilmaz, O., & Turkoglu, I. (2014). The Investigation of Some

- 695 Bioactive Compounds and Antioxidant Properties of Hawthorn (*Crataegus monogyna* subsp.
696 *monogyna* jacq.). *Journal of Intercultural Ethnopharmacology*.
697 <https://doi.org/10.5455/jice.20140120103320>
- 698 Khaleel, S. M. J., & Haddadin, M. S. Y. (2013). The enhancement of hawthorn leaf extracts on the
699 growth and production of short chain fatty acids of two probiotic bacteria. *Pakistan Journal of*
700 *Nutrition*. <https://doi.org/10.3923/pjn.2013.144.149>
- 701 Kinder, J. M., Then, J. E., Hansel, P. M., Molinero, L. L., & Bruns, H. A. (2014). Long-term
702 repeated daily use of intragastric gavage hinders induction of oral tolerance to ovalbumin in
703 mice. *Comparative Medicine*, 64(5), 369–376.
- 704 Kruidenier, L., Kuiper, I., van Duijn, W., Mieremet-Ooms, M. A. C., van Hogezaand, R. A., Lamers,
705 C. B. H. W., & Verspaget, H. W. (2003). Imbalanced secondary mucosal antioxidant response
706 in inflammatory bowel disease. *Journal of Pathology*. <https://doi.org/10.1002/path.1408>
- 707 Kumar, D., Arya, V., Bhat, Z. A., Khan, N. A., & Prasad, D. N. (2012). The genus *Crataegus*:
708 Chemical and pharmacological perspectives. In *Brazilian Journal of Pharmacognosy*.
709 <https://doi.org/10.1590/S0102-695X2012005000094>
- 710 Lacucci, M., De Silva, S., & Ghosh, S. (2010). Mesalazine in inflammatory bowel disease: A trendy
711 topic once again? In *Canadian Journal of Gastroenterology*.
712 <https://doi.org/10.1155/2010/586092>
- 713 Lamaison, J. L., & Carnat, A. (1990). Teneur en principaux flavonoides des fleurs et des feuilles de
714 *Crataegus monogyna* jacq. et de *Crataegus laevigata* (poiret) dc. (Rosaceae). *Pharmaceutica*
715 *Acta Helvetiae*.
- 716 Lasa, J., Correa, G., Fuxman, C., Garbi, L., Linares, M. E., Lubrano, P., Rausch, A., Toro, M.,
717 Yantorno, M., Zubiaurre, I., Peyrin-Biroulet, L., & Olivera, P. (2020). Treatment Adherence in
718 Inflammatory Bowel Disease Patients from Argentina: A Multicenter Study. *Gastroenterology*
719 *Research and Practice*, 2020, 4060648. <https://doi.org/10.1155/2020/4060648>
- 720 LeBlanc, J. G., del Carmen, S., Miyoshi, A., Azevedo, V., Sesma, F., Langella, P., Bermúdez-

- 721 Humaran, L. G., Watterlot, L., Perdigon, G., & de Moreno de LeBlanc, A. (2011). Use of
722 superoxide dismutase and catalase producing lactic acid bacteria in TNBS induced Crohn's
723 disease in mice. *Journal of Biotechnology*, 151(3), 287–293.
724 [https://doi.org/https://doi.org/10.1016/j.jbiotec.2010.11.008](https://doi.org/10.1016/j.jbiotec.2010.11.008)
- 725 Leite, A. V., Malta, L. G., Riccio, M. F., Eberlin, M. N., Pastore, G. M., & Maróstica Júnior, M. R.
726 (2011). Antioxidant potential of rat plasma by administration of freeze-dried jaboticaba peel
727 (Myrciaria jaboticaba Vell Berg). *Journal of Agricultural and Food Chemistry*, 59(6), 2277–
728 2283. <https://doi.org/10.1021/jf103181x>
- 729 Liu, F., Zhang, X., & Ji, Y. (2020). Total Flavonoid Extract from Hawthorn (*Crataegus pinnatifida*)
730 Improves Inflammatory Cytokines-Evoked Epithelial Barrier Deficit. *Medical Science*
731 *Monitor : International Medical Journal of Experimental and Clinical Research*.
732 <https://doi.org/10.12659/MSM.920170>
- 733 Lopez-Castejon, G., & Brough, D. (2011). Understanding the mechanism of IL-1 β secretion. In
734 *Cytokine and Growth Factor Reviews*. <https://doi.org/10.1016/j.cytogfr.2011.10.001>
- 735 Malekinejad, H., Shafie-Irannejad, V., Hobbenaghi, R., Tabatabaie, S. H., & Moshtaghion, S. M.
736 (2013). Comparative protective effect of hawthorn berry hydroalcoholic extract, atorvastatin,
737 and mesalamine on experimentally induced colitis in rats. *Journal of Medicinal Food*.
738 <https://doi.org/10.1089/jmf.2012.2672>
- 739 Martino, E., Collina, S., Rossi, D., Bazzoni, D., Gaggeri, R., Bracco, F., & Azzolina, O. (2008).
740 Influence of the extraction mode on the yield of hyperoside, vitexin and vitexin-2-O-
741 rhamnoside from *Crataegus monogyna* Jacq. (Hawthorn). *Phytochemical Analysis*.
742 <https://doi.org/10.1002/pca.1081>
- 743 Maurer, Luana H., Cazarin, C. B. B., Quatrin, A., Minuzzi, N. M., Nichelle, S. M., Lamas, C. de A.,
744 Cagnon, V. H. A., Morari, J., Velloso, L. A., Maróstica Júnior, M. R., & Emanuelli, T. (2020).
745 Grape peel powder attenuates the inflammatory and oxidative response of experimental colitis
746 in rats by modulating the NF- κ B pathway and activity of antioxidant enzymes. *Nutrition*

- 747 *Research*. <https://doi.org/10.1016/j.nutres.2020.01.006>
- 748 Maurer, Luana Haselein, Cazarin, C. B. B., Quatrin, A., Nichelle, S. M., Minuzzi, N. M., Teixeira,
749 C. F., Manica da Cruz, I. B., Maróstica Júnior, M. R., & Emanuelli, T. (2019). Dietary fiber
750 and fiber-bound polyphenols of grape peel powder promote GSH recycling and prevent
751 apoptosis in the colon of rats with TNBS-induced colitis. *Journal of Functional Foods*.
752 <https://doi.org/10.1016/j.jff.2019.103644>
- 753 Moura, F. A., de Andrade, K. Q., dos Santos, J. C. F., Araújo, O. R. P., & Goulart, M. O. F. (2015).
754 Antioxidant therapy for treatment of inflammatory bowel disease: Does it work? In *Redox*
755 *Biology*. <https://doi.org/10.1016/j.redox.2015.10.006>
- 756 Nascimento, R. de P. do, Lima, A. V., Oyama, L. M., Paiotti, A. P. R., Cardili, L., Martinez, C. A.
757 R., Pereira, J. A., Silva, M. F., Garofolo, I. C., Silveira, V. L. F., & Caperuto, L. C. (2020).
758 Extra-virgin olive oil and flaxseed oil have no preventive effects on dss-induced acute
759 ulcerative colitis. *Nutrition*, 110731. <https://doi.org/10.1016/j.nut.2020.110731>
- 760 Nascimento, R. de P. do, Machado, A. P. da F., Galvez, J., Cazarin, C. B. B., & Maróstica Junior,
761 M. R. (2020). Ulcerative colitis: Gut microbiota, immunopathogenesis and application of
762 natural products in animal models. *Life Sciences*, 118129.
763 <https://doi.org/https://doi.org/10.1016/j.lfs.2020.118129>
- 764 Nathan, M. (1999). The Complete German Commission E Monographs: Therapeutic Guide to
765 Herbal Medicines. *Annals of Internal Medicine*. [https://doi.org/10.7326/0003-4819-130-5-](https://doi.org/10.7326/0003-4819-130-5-199903020-00024)
766 [199903020-00024](https://doi.org/10.7326/0003-4819-130-5-199903020-00024)
- 767 Ng, S. C., Shi, H. Y., Hamidi, N., Underwood, F. E., Tang, W., Benchimol, E. I., Panaccione, R.,
768 Ghosh, S., Wu, J. C. Y., Chan, F. K. L., Sung, J. J. Y., & Kaplan, G. G. (2017). Worldwide
769 incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic
770 review of population-based studies. *The Lancet*, 390(10114), 2769–2778.
771 [https://doi.org/10.1016/S0140-6736\(17\)32448-0](https://doi.org/10.1016/S0140-6736(17)32448-0)
- 772 Ngoc, P. C., Leclercq, L., Rossi, J. C., Desvignes, I., Hertzog, J., Fabiano-Tixier, A. S., Chemat, F.,

- 773 Schmitt-Koppin, P., & Couet, H. (2019). Optimizing water-based extraction of bioactive
774 principles of hawthorn: From experimental laboratory research to homemade preparations.
775 *Molecules*. <https://doi.org/10.3390/molecules24234420>
- 776 Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by
777 thiobarbituric acid reaction. *Analytical Biochemistry*. [https://doi.org/10.1016/0003-](https://doi.org/10.1016/0003-2697(79)90738-3)
778 [2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
- 779 Ou, B., Chang, T., Huang, D., & Prior, R. L. (2013). Determination of total antioxidant capacity by
780 oxygen radical absorbance capacity (ORAC) using fluorescein as the fluorescence probe: First
781 action 2012.23. *Journal of AOAC International*. <https://doi.org/10.5740/jaoacint.13-175>
- 782 Paiotti, A. P. R., Neto, R. A., Marchi, P., Silva, R. M., Pazine, V. L., Noguti, J., Pastrelo, M. M.,
783 Gollücke, A. P. B., Miszputen, S. J., & Ribeiro, D. A. (2013). The anti-inflammatory potential
784 of phenolic compounds in grape juice concentrate (G8000™) on 2,4,6-trinitrobenzene
785 sulphonic acid-induced colitis. *British Journal of Nutrition*.
786 <https://doi.org/10.1017/S000711451300007X>
- 787 Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of*
788 *Nutritional Science*, 5, e47. <https://doi.org/10.1017/jns.2016.41>
- 789 Parkar, S. G., Trower, T. M., & Stevenson, D. E. (2013). Fecal microbial metabolism of
790 polyphenols and its effects on human gut microbiota. *Anaerobe*.
791 <https://doi.org/10.1016/j.anaerobe.2013.07.009>
- 792 Patel, P. P., & Trivedi, N. D. (2017). Effect of karanjin on 2,4,6-trinitrobenzenesulfonic acid-
793 induced colitis in Balb/c mice. *Indian Journal of Pharmacology*.
794 https://doi.org/10.4103/ijp.IJP_234_15
- 795 Pérez, S., Taléns-Visconti, R., Rius-Pérez, S., Finamor, I., & Sastre, J. (2017). Redox signaling in
796 the gastrointestinal tract. In *Free Radical Biology and Medicine*.
797 <https://doi.org/10.1016/j.freeradbiomed.2016.12.048>
- 798 Peterson, C. G. B., Sangfelt, P., Wagner, M., Hansson, T., Lettesjö, H., & Carlson, M. (2007). Fecal

- 799 levels of leukocyte markers reflect disease activity in patients with ulcerative colitis.
800 *Scandinavian Journal of Clinical and Laboratory Investigation*.
801 <https://doi.org/10.1080/00365510701452838>
- 802 Pieper, C., Haag, S., Gesenhues, S., Holtmann, G., Gerken, G., & Jöckel, K.-H. (2009). Guideline
803 adherence and patient satisfaction in the treatment of inflammatory bowel disorders--an
804 evaluation study. *BMC Health Services Research*, 9, 17. [https://doi.org/10.1186/1472-6963-9-](https://doi.org/10.1186/1472-6963-9-17)
805 17
- 806 Porter, L. J., Hrstich, L. N., & Chan, B. G. (1985). The conversion of procyanidins and
807 prodelphinidins to cyanidin and delphinidin. *Phytochemistry*. [https://doi.org/10.1016/S0031-](https://doi.org/10.1016/S0031-9422(00)94533-3)
808 9422(00)94533-3
- 809 Rauf, A., Imran, M., Abu-Izneid, T., Iahtisham-Ul-Haq, Patel, S., Pan, X., Naz, S., Silva, A.
810 [Sanches, Saeed, F., & Suleria], H. A. [Rasul. (2019). Proanthocyanidins: A comprehensive
811 review. *Biomedicine & Pharmacotherapy*, 116, 108999.
812 <https://doi.org/https://doi.org/10.1016/j.biopha.2019.108999>
- 813 Sánchez-Fidalgo, S., Villegas, I., Cárdeno, A., Talero, E., Sánchez-Hidalgo, M., Motilva, V., &
814 Alarcón de la Lastra, C. (2010). Extra-virgin olive oil-enriched diet modulates DSS-colitis-
815 associated colon carcinogenesis in mice. *Clinical Nutrition*, 29(5), 663–673.
816 <https://doi.org/10.1016/j.clnu.2010.03.003>
- 817 Simpson, B. ., Aryee, A. N., & Toldrá, F. (2019). *Byproducts from Agriculture and Fisheries:*
818 *Adding Value for Food, Feed, Pharma and Fuels*. Wiley.
- 819 Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-
820 Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16(3), 144–
821 158. <https://www.ajevonline.org/content/16/3/144>
- 822 Therrien, A., Chapuy, L., Bsat, M., Rubio, M., Bernard, G., Arslanian, E., Orlicka, K., Weber, A.,
823 Panzini, B.-P., Dorais, J., Bernard, E.-J., Soucy, G., Bouin, M., & Sarfati, M. (2019).
824 Recruitment of activated neutrophils correlates with disease severity in adult Crohn's disease.

- 825 *Clinical and Experimental Immunology*, 195(2), 251–264. <https://doi.org/10.1111/cei.13226>
- 826 Ungaro, R., Mehandru, S., Allen, P. B., Peyrin-biroulet, L., & Colombel, J. (2017). Ulcerative
827 colitis. *Lancet*, 389(10080). [https://doi.org/10.1016/S0140-6736\(16\)32126-2](https://doi.org/10.1016/S0140-6736(16)32126-2)
- 828 Vasilyeva, E., Abdulkhakov, S., Cherepnev, G., Martynova, E., Mayanskaya, I., Valeeva, A.,
829 Abdulkhakov, R., Safina, D., Khaiboullina, S., & Rizvanov, A. (2016). Serum cytokine
830 profiles in children with Crohn's disease. *Mediators of Inflammation*.
831 <https://doi.org/10.1155/2016/7420127>
- 832 Walker, A. F., Marakis, G., Simpson, E., Hope, J. L., Robinson, P. A., Hassanein, M., & Simpson,
833 H. C. R. (2006). Hypotensive effects of hawthorn for patients with diabetes taking prescription
834 drugs: A randomised controlled trial. *British Journal of General Practice*.
- 835 Weber, R. W. (2010). On the Cover – Hawthorn. *Annals of Allergy, Asthma and Immunology*.
836 <https://doi.org/10.1016/j.anai.2010.09.018>
- 837 Wei, W., Ying, X., Zhang, W., Chen, Y., Leng, A., Jiang, C., & Liu, J. (2014). Effects of vitexin-
838 2"-O-rhamnoside and vitexin-4"-O-glucoside on growth and oxidative stress-induced cell
839 apoptosis of human adipose-derived stem cells. *The Journal of Pharmacy and Pharmacology*,
840 66(7), 988–997. <https://doi.org/10.1111/jphp.12225>
- 841 Wéra, O., Lancellotti, P., & Oury, C. (2016). The Dual Role of Neutrophils in Inflammatory Bowel
842 Diseases. *Journal of Clinical Medicine*. <https://doi.org/10.3390/jcm5120118>
- 843 Winterbourn, C. C., Hawkins, R. E., Brian, M., & Carrell, R. W. (1975). The estimation of red cell
844 superoxide dismutase activity. *The Journal of Laboratory and Clinical Medicine*.
845 <https://doi.org/10.5555/uri:pii:0022214375904394>
- 846 Yoshikawa, T., Naito, Y., Kishi, A., Tomii, T., Kaneko, T., Inuma, S., Ichikawa, H., Yasuda, M.,
847 Takahashi, S., & Kondo, M. (1993). Role of active oxygen, lipid peroxidation, and
848 antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats.
849 *Gut*. <https://doi.org/10.1136/gut.34.6.732>
- 850 Zapatero, J. M. (1999). Selections from current literature: Effects of Hawthorn on the

- 851 cardiovascular system. *Family Practice*. <https://doi.org/10.1093/fampra/16.5.534>
- 852 Zatorski, H., Sałaga, M., Zielińska, M., Piechota-Polańczyk, A., Owczarek, K., Kordek, R.,
853 Lewandowska, U., Chen, C., & Fichna, J. (2015). Experimental colitis in mice is attenuated by
854 topical administration of chlorogenic acid. *Naunyn-Schmiedeberg's Archives of*
855 *Pharmacology*. <https://doi.org/10.1007/s00210-015-1110-9>
- 856 Zhao, G., Nyman, M., & Jonsson, J. (2006). Rapid determination of short-chain fatty acids in
857 colonic contents and faeces of humans and rats by acidified water-extraction and direct-
858 injection gas chromatography. *Biomedical Chromatography : BMC*, 20, 674–682.
859 <https://doi.org/10.1002/bmc.580>
- 860 Zhi, C. Y., Lv, Z., Kong, N., Chen, Y., Wang, Z. H., & Gao, H. C. (2017). Effect on various organs
861 in TNBS-induced ulcerative colitis. *Biomedical Research (India)*.
- 862 Zhou, Y., Zhou, L., Ruan, Z., Mi, S., Jiang, M., Li, X., Wu, X., Deng, Z., & Yin, Y. (2016).
863 Chlorogenic acid ameliorates intestinal mitochondrial injury by increasing antioxidant effects
864 and activity of respiratory complexes. *Bioscience, Biotechnology and Biochemistry*.
865 <https://doi.org/10.1080/09168451.2015.1127130>
- 866 Zhu, X. X., Li, L. Da, Liu, J. X., Liu, Z. Y., & Ma, X. Y. (2006). Effect of vitexia-rhamnoside (V-
867 R) on vasomotor factors expression of endothelial cell. *Zhongguo Zhongyao Zazhi*.
- 868
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TABLE 1. HISTOLOGICAL GRADING OF COLITIS.

Score	Leukocyte infiltration	Inflammation extent	Crypt damage
0	None	None	None
1	Discrete	Mucosa	1/3 damaged (basal)
2	Moderate	Mucosa and submucosa	2/3 damaged (basal)
3	Severe	Transmural	Only the surface of the epithelium is intact
4	-	-	Loss of crypt and epithelium

Area involved in percentage (score): 1–25 (1), 26–50 (2), 51–75 (3), 76–100 (4)

878 For each category of the score (leukocyte infiltration, inflammation extent, and crypt damage),
 879 points were multiplied by a factor of involvement of the colonic tissue. The sum of the three
 880 categories adds up to the total score of each section (Dieleman et al., 1998).

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Table 2. Peak identification of the main compounds detected by UHPLC-ESI-MS.

Peak	Retention time (min)	λ_{\max} (nm)	$[M+H]^+$	Other ions in the spectrum	Identified compound
1	2.71	204, 218, 260	288		Cyanidin
2	3.73	218, 236, 324	355	377, 711	5-O-Caffeoylquinic acid
3	7.49	219, 238, 325	355	377, 711	Chlorogenic acid (3-O-caffeoylquinic acid)
4	9.1	227, 280	579	427, 289	Procyanidin B2
5	9.45	224, 279	291	147, 139, 123	Epicatechin
6	12.24	280	867	579	Procyanidin C1
7	13.37	219, 280	1155	287, 413, 575	Cinnamtannin A2
8	15.42	216, 269, 338	579	433, 313	Vitexin 2-O-rhamnoside
9	15.68	206, 262, 348	415	397, 367, 283	Pinnatifinoside A
10	16.13	220, 256, 353	465	303	Hyperoside
11	16.52	202, 257, 353	303	621	Isoquercetin
12	19.85	268, 337	433	621	Apigenin-C-hexoside

895 λ_{\max} is the local maximum absorbance in the UV spectrum. $[M+H]^+$ provided the m/z value of the
896 precursor ion. Other ions provided the m/z value from fragments detected in the mass spectrometry.

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Table 3. Macroscopic and microscopic parameters.

Largest necrotic lesion					
Parameter	<i>C</i>	<i>CC</i>	<i>H</i>	<i>HC</i>	<i>MC</i>
Width (cm)	0a	2.04 ± 0.31b	0a	1.05 ± 0.28b	1.13b ± 0.27b
Length (cm)	0a	2.38 ± 0.17b	0a	0.96 ± 0.22c	0.92 ± 0.27c
Area (width x length, cm ²)	0a	5.02 ± 1.13b	0a	1.31 ± 0.53a	0.72 ± 0.27a
Lesion/colon length (%)	0a	11.51 ± 1.40b	0a	4.67 ± 1.20a	4.83 ± 1.75a
Histology*					
Leukocyte infiltration	0a	8.20 ± 1.80b	0a	7.33 ± 0.98b	4.85 ± 0.73b
Inflammation extent	0a	7.40 ± 1.69b	0a	7.00 ± 1.12b	5.14 ± 0.59b
Crypt damage	0a	8.00 ± 2.21b	0a	6.00 ± 2.11b	4.74 ± 0.60b
TOTAL (sum)	0a	23.60 ± 5.41b	0a	16.40 ± 1.03b**	14.71 ± 1.79b

906 *C*: control, *CC*: control colitis, *H*: hawthorn, *HC*: hawthorn colitis, *MC*: mesalamine colitis.

907 Results are presented as mean ± SEM. One-way ANOVA followed by Tukey. Different letters
 908 indicate statistical significance ($p < 0.05$). *Score calculation: each parameter (crypt damage,
 909 inflammation extent, and leukocyte infiltration) was multiplied by the factor of involvement of the
 910 mucosa, and the total is the sum of the three results (Dieleman et al., 1998). **The sum of this
 911 group was not 20.33 ± 4.21 , as the sum of the three parameters suggests (sum of the scores of
 912 leukocyte infiltration, inflammation extent, and crypt damage); this happened due to an outlier only
 913 found in the statistical analysis of the TOTAL.

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Table 4. Antioxidant capacity of the serum, inflammatory profile, and activity of**concentration of oxidative metabolism enzymes.**

Antioxidant analyses (serum)					
Parameter	C	CC	H	HC	MC
FRAP	0.62 ±	0.55 ±	0.60 ±	0.56 ±	0.59 ±
(µmol Trolox/mL)	0.06a	0.04a	0.05a	0.01a	0.06a
ORAC	4.31 ±	3.82 ±	7.26 ±	5.63 ±	4.04 ±
(µmol Trolox/mL)	0.71a	0.81a	2.08a	0.47a	0.65a
Inflammatory profile (colon and serum)					
MPO (colon)	9.40 ±	39.80 ±	9.80 ±	11.67 ±	8.66 ±
(U/g protein)	0.50a	11.27b	1.49a	2.74a	0.61a
IL-1β (colon)	111.20 ±	529.90 ±	81.34 ±	271.30 ±	208.00 ±
(ng/g protein)	3.25a	127.30b	14.20a	39.32a	39.78a
IL-1β (serum)	0.40 ±	0.44 ±	0.44 ±	0.47 ±	0.34 ±
(ng/mL)	0.04a	0.08a	0.06a	0.09a	0.03a
TNF-α (colon)	86.27 ±	77.64 ±	85.73 ±	106.50 ±	105.80 ±
(ng/g protein)	2.37a	16.17a	4.25a	15.38a	19.70a
TNF-α (serum)	0.07 ±	0.17 ±	0.06 ±	0.13 ±	0.08 ±
(ng/mL)	0.01a	0.06a	0.01a	0.06a	0.01a
Oxidative metabolism enzymes (colon)					
CAT	87.60 ±	211.00 ±	56.00 ±	86.50 ±	209.90 ±
(U/g protein)	10.49a	33.48b	13.33a	20.63a	21.85b
GR (consumed	106.20 ±	41.60 ±	198.00 ±	324.50 ±	58.71 ±
NADPH/min/g	19.7ab	5.11a	76.51bc	19.47c	9.38a
protein)					

SOD	144.40 ±	56.00 ±	142.80 ±	105.00 ±	54.67 ±
(SOD/g protein)	45.57 a	16.83 a	43.34 a	32.83 a	4.81 a
TBARS (nmol	66.78 ±	77.58 ±	37.66 ±	68.30 ±	56.41 ±
MDA/g protein)	12.42 a	24.24 a	10.84 a	10.49 a	8.02 a
GSH (nmol GSH/mg	14.25 ±	9.39 ±	16.70 ±	11.22 ±	13.74 ±
protein)	2.85 a	4.06 a	2.08 a	2.21 a	1.94 a

921 *C*: control, *CC*: control colitis, *H*: hawthorn, *HC*: hawthorn colitis, *MC*: mesalamine colitis. FRAP:
922 ferric reducing antioxidant power, ORAC: oxygen-radical absorbing capacity. CAT: catalase, GR:
923 glutathione reductase, MPO: myeloperoxidase, SOD: superoxide dismutase, TBARS: thiobarbituric
924 acid reactive substances, MDA: malondialdehyde, GSH: glutathione. IL-1 β : interleukin-1beta,
925 TNF- α : tumor necrosis factor-alpha. Results are presented as mean \pm SEM. One-way ANOVA
926 followed by Tukey. Different letters indicate statistical significance ($p < 0,05$).

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943 **Fig. 1. UHPLC profile of the main identified polyphenols in the hawthorn tea**

944 Peak or relative area (%) identification: 1: Cyanidin, 2: 5-*O*-caffeoylquinic acid, 3: chlorogenic
945 acid, 4: procyanidin B2, 5: epicatechin, 6: procyanidin C1, 7: cinnamtannin A2, 8: vitexin-2-*O*-
946 rhamnoside, 9: pinnatifinose A, 10: hyperoside, 11: isoquercetin, 12: apigenin C-hexoside. The
947 analysis was performed in triplicate. **A.** Peak profile of compounds from hawthorn tea by UV
948 monitoring at 273 nm. **B.** Relative peak area (%) of compounds peaks from hawthorn tea. The
949 relative area was calculated by dividing the peak area of each component by the sum of the peak
950 area of the 12 identified components.

951

952 **Fig. 2. Body weight, colon weight/length ratio, and appearance of necrotic lesions**

953 *C: control, CC: control colitis, H: hawthorn, HC: hawthorn colitis, MC: mesalamine colitis.*

954 Results are presented as mean \pm SEM. One-way or Two-way ANOVA followed by Tukey.

955 Different letters indicate statistical significance ($p < 0,05$). **A.** Body weight alteration (%) after colitis
956 induction (days 15 to 21). The calculation was determined in relation to day 14. **B.** Colon
957 weight/colon length (g/cm) ratio as an inflammatory index. **C.** Photographic representation of the
958 necrotic lesions on the colonic mucosa of rats with colitis.

959

960 **Fig. 3. Photographic representation of the score of stool consistency**

961 **A.** Score 0 - Normal stools. **B.** Score 1 - Soft pellets not adhering to the anus. **C.** Score 2 - Very soft
962 pellets adhering to the anus. **D.** Score 3 - Liquid stool; wet anus. Score based on Gommeaux et al.
963 (Gommeaux et al., 2007) and photographic scheme based on Nascimento et al. (Nascimento, Lima,
964 et al., 2020).

965

966 **Fig. 4. Photographic representation of the score of bleeding**

967 **A.** Score 0 – NO bleeding. **B.** Score 1 - Small spots of blood in stool; dry anal region. **C.** Score 2 -
 968 Large spots of blood in stool; blood appears through the anal orifice. **D.** Score 3 - Deep red stool;
 969 blood spreads largely around the anus. Score based on Gommeaux et al. (Gommeaux et al., 2007)
 970 and photographic scheme based on Nascimento et al. (Nascimento, Lima, et al., 2020).

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972 **Fig. 5. Photographic representation of the distal colon histology**

973 Hematoxylin and eosin stain. Settings: 10x/0.25 magnification, 6.31x/0.25 AxioCam ICc5 (Zeiss,
 974 Germany). Scale: 100 μ m. *C: control, CC: control colitis, H: hawthorn, HC: hawthorn colitis, MC:*
 975 *mesalamine colitis.*

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977 **Fig. 6. Heatmap correlation matrix**

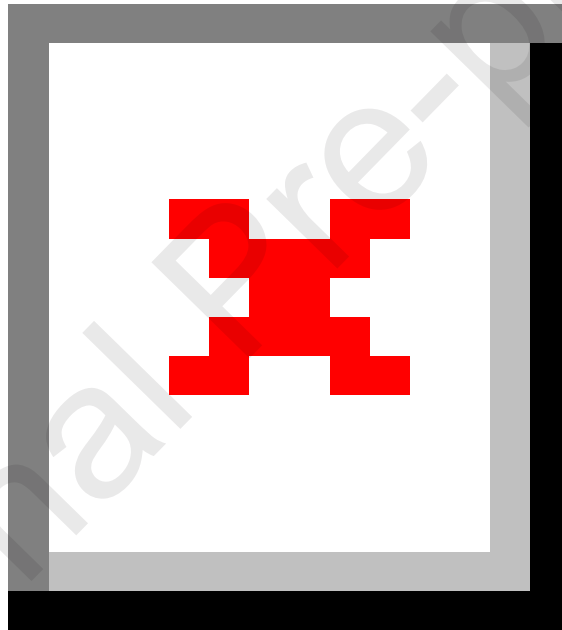
978 Pearson's correlation analysis. Correlations considered high (values between 0.7 and 1 or -0.7 and -
 979 1) are marked with an asterisk (*). Parameters: WEIGHT %: body weight alteration on euthanasia;
 980 DAI DAY 1: Disease Activity Index after one day of the administration of 2,4,6-trinitrobenzene
 981 sulfonic (peak day of symptoms); W/L COLON: colon weight/length ratio; LESION Wi: width of
 982 the largest necrotic lesion; LESION L: length of the largest necrotic lesion; LESION A: area of the
 983 largest necrotic lesion; LESION %: the proportion of the colon affected by the largest necrotic
 984 lesion; HISTOLOGY T: total score of the histology; HISTOLOGY LI: score of the leukocyte
 985 infiltration; HISTOLOGY IE: score of the inflammation extent; HISTOLOGY CD: score of the
 986 crypt damage; CAT: catalase levels in the colon; GR: glutathione reductase levels in the colon;
 987 MPO: myeloperoxidase levels in the colon; SOD: superoxide dismutase levels in the colon; IL-1 β :
 988 interleukin-1beta levels in the colon.

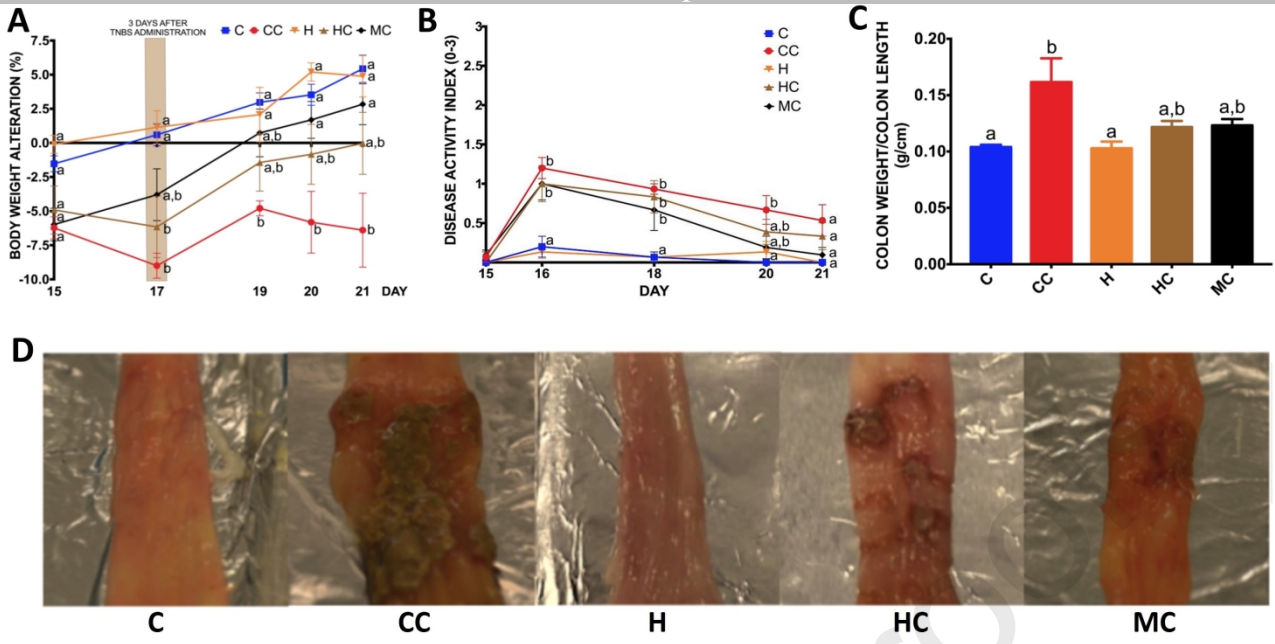
989 **HIGHLIGHTS**

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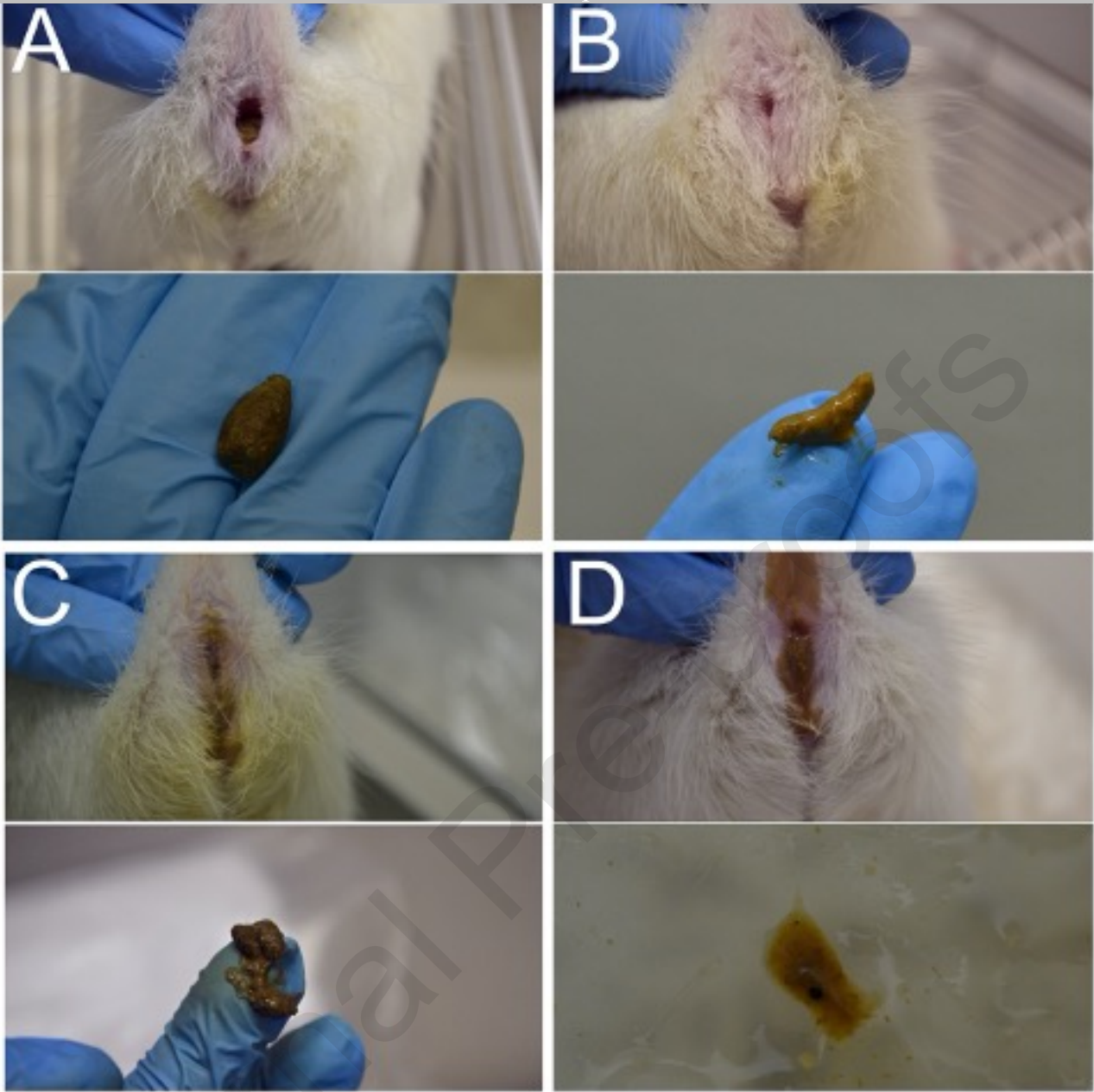
- 991 • **A tea from the leaves and flowers of hawthorn** is rich in flavonoids, especially
 992 vitexin-2-O-rhamnoside.

- 993
- mesalamine and hawthorn tea have positive healing effects in rats with colitis.
- 994
- Hawthorn tea reduces the length and area of the brownish necrotic lesions.
- 995
- Hawthorn tea diminishes the levels of the inflammatory markers MPO and IL-1 β .
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- Hawthorn tea regulates the activity of the oxidative stress enzymes CAT and GR.
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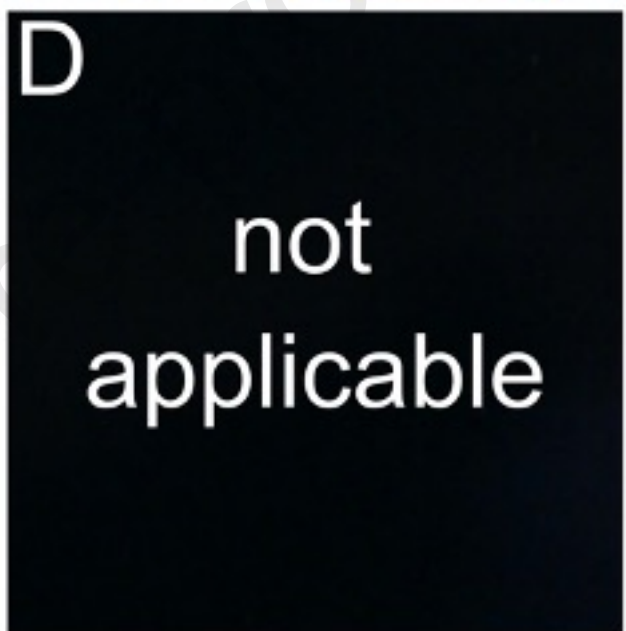




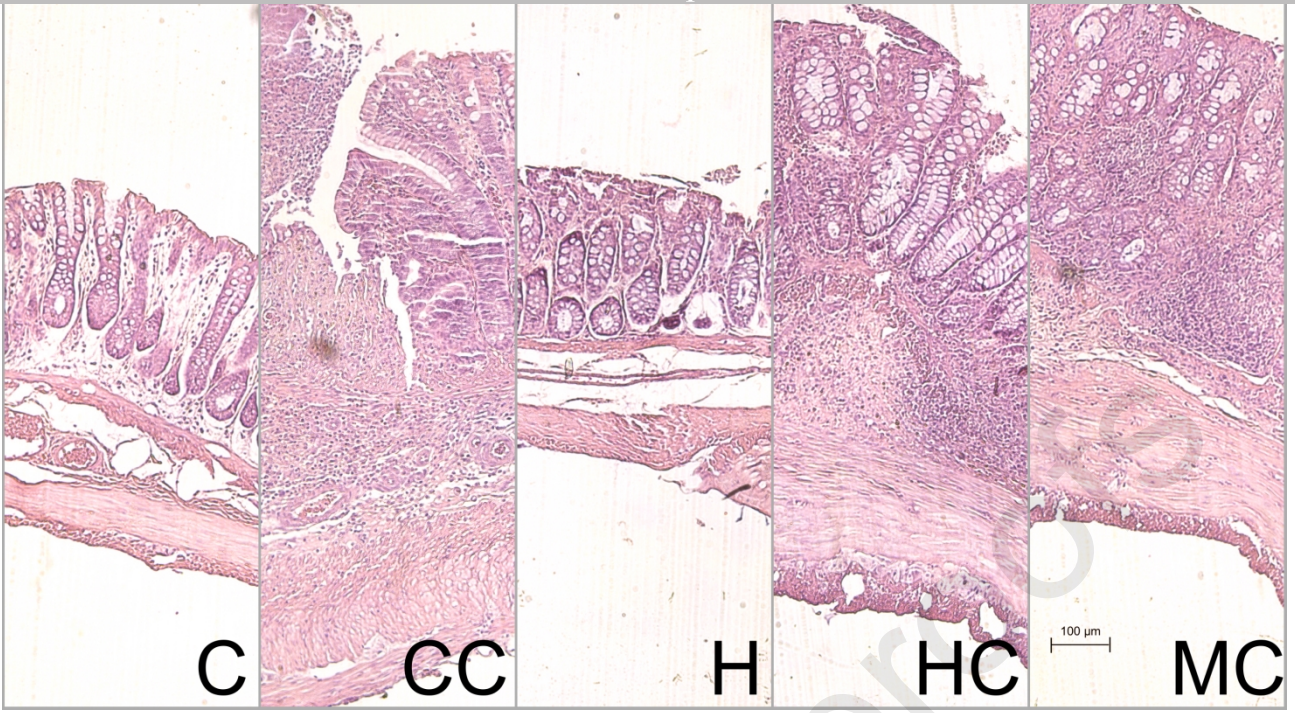
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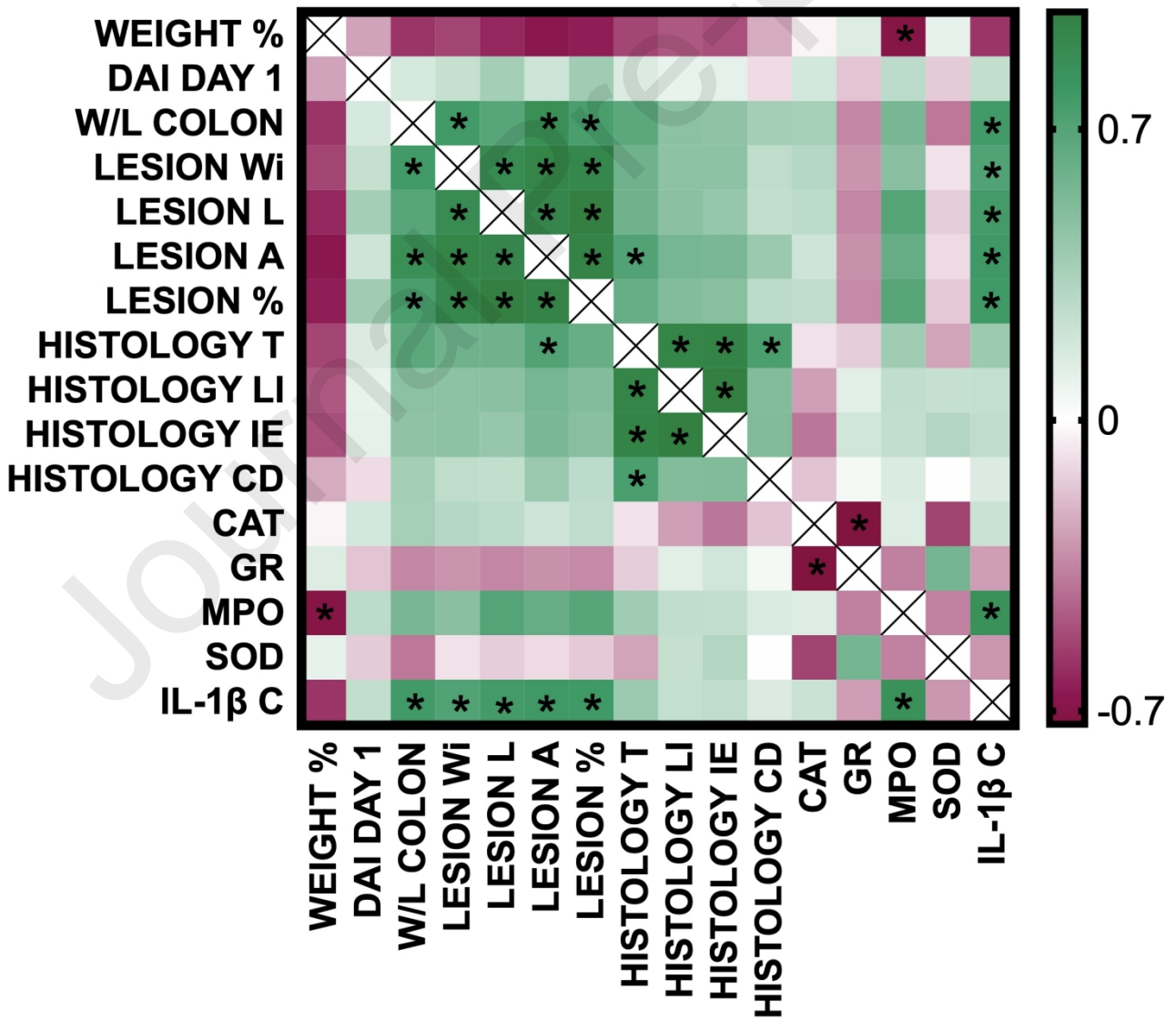
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HAWTHORN PLANT



Crataegus oxyacantha: rich in total polyphenols, flavonoids, and proanthocyanidins

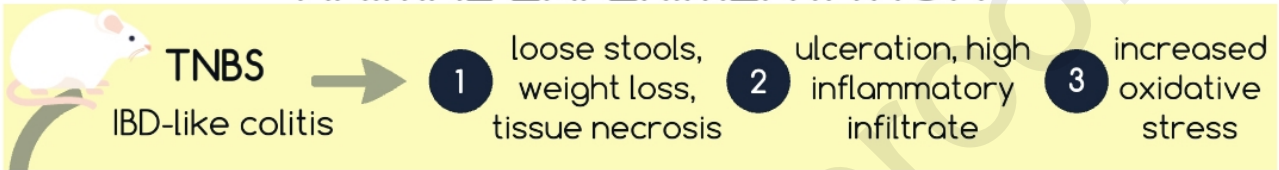


Used to treat chronic congestive heart failure. Evidence for **intestinal diseases** is still poorly known.

INFLAMMATORY BOWEL DISEASES



ANIMAL EXPERIMENTATION



PREVENTIVE EFFECTS



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