Journal of Research in Pharmacy

ResearchArticle

www.jrespharm.com

Experimental pharmacology assessment of *Agave brittoniana* subsp. *brachypus* (Trel.) in ulcerative colitis

Yisel GONZÁLEZ-MADARIAGA[1]*, Dayli SANTIESTFBAN-MUÑOZ[1], Leisy NIETO-REYES[2], María L. GARCÍA-GÓMEZ[1], José O. GUERRA DE LEÓN[3]

1 Experimental Toxicology Unit, University of Medical Sciences, Villa Clara, Cuba.
2 Department of Pharmacy, Faculty of Chemistry-Pharmacy, Central University "Marta Abreu" of Las Villas, Santa Clara, Villa Clara, Cuba.
3 Department of Chemistry, Faculty of Chemistry-Pharmacy, Central University "Marta Abreu" of Las Villas, Santa Clara, Villa Clara, Cuba
* Corresponding Author. E-mail: yiselmadariaga@gmail.com (Y.G.M.); Tel. +53-532-159-16.

Received: 24 August 2023 / Revised: 12 February 2024 / Accepted: 14 February 2024

ABSTRACT: Ulcerative colitis is a chronic inflammatory bowel disease with an unknown cause. It is characterized by damage to the colonic mucosa, which often results in symptoms such as diarrhea, abdominal pain, and weight loss, ultimately leading to a reduced quality of life. Treatment aims to control inflammation, reduce symptoms, and minimize fluid and nutrient losses. Currently, there is no known cure for ulcerative colitis. The study was carried out to investigate the anti-inflammatory potential of the hydroalcoholic extract (HAE) and the butanolic fraction (FB) from *Agave brittoniana* subsp. *brachypus (*Trel.) on acetic acid-induced colitis in rats. Ulcerative colitis was induced in Sprague Dawley rats using 2.5% acetic acid. Three dose levels for both HAE (150-300-600 mg/kg) and BF (25-50-100 mg/kg) were evaluated. The study recorded the variation of body weight, macroscopic and microscopic colonic damage index, and colonic myeloperoxidase. Body weight loss was lower in all treated groups compared to the untreated group. Similarly, there were fewer signs of necrosis and tissue damage in the colon in the groups treated with HAE and BF. *Agave brittoniana* T exhibited an effective anti-inflammatory effect in an experimental model of ulcerative colitis, indicating its potential as a natural treatment for the condition.

KEYWORDS: colitis; agave; myeloperoxidase; *Sprague-Dawley*.

1. INTRODUCTION

Ulcerative colitis (UC) is a chronic and recurrent inflammatory process that affects the mucosa and colorectal submucosa, with diffuse and continuous distribution. It causes diarrhea with blood, abdominal pain, and sometimes fever. Patients with UC have an increased risk of developing colorectal cancer, which is the second leading cause of cancer-related deaths worldwide. Individuals with this disease experience a diminished quality of life as a result of its chronic nature and the lack of a curative treatment. [1, 2]. This has made it necessary to search for natural treatment alternatives.

The genus *Agave* L (Asparagaceae) consists of about 250 species can grow primarily in the arid and semiarid regions [3]. Its distribution is extends from the southern United States to Colombia and Venezuela including the Caribbean islands [4, 5]. Nearly 84% of agave species are endemic to Mexico, with the United States, Cuba, and Guatemala also having a significant number of agave taxa in the area [6, 7]. About 14-16 species are found in Cuba, with a high level of endemism (60%) [8]. Agaves are now widespread in South Africa, the Mediterranean basin, Indonesia, the Philippines, and other semiarid regions around the world [9].

For centuries, this genus has been used for food, fibres, beverages, and ornamentals [10, 11]. Additionally, agave species have historically been used in indigenous medicine to treat anti-inflammatory processes, arthritis, and fever [12, 13]. Screening the phytochemical composition of this genus has identified many species as potential sources of drugs for treating cancer, diabetes, and obesity [14-16]. Furthermore, many agave species contain steroidal saponins in their secondary metabolites. Depending on their structure, these saponins can have various pharmacological effects, including anti-inflammatory properties. [17, 18]. Previous studies have demonstrated the anti-inflammatory activity of various species of agave, including *Agave angustifolia* [19], *Agave americana* [20] and *Agave sisalana* [21].

How to cite this article: González-Madariaga Y, SantıestFBan-Muñoz D, Nıeto-Reyes L, García-Gómez ML, Guerra de León JO. Experimental pharmacology assessment of *Agave brittoniana* subsp. *brachypus* (Trel.) in ulcerative colitis. J ResPharm. 2024; 28(6): 1963-1973.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.870

In Cuba, research has primarily focused on certain agave species with significant pharmaceutical importance. One such species is the endemic *Agave brittoniana* subsp. *brachypus*, found in the central region, which was first described by William Trelease in 1913 [22] and later by Álvarez de Zayas in 1995 [23].

Agave brittoniana subsp. *brachypus* (Trel.) is known for its high content of steroidal saponins [24, 25]. Its composition also includes metabolites such as waxes, long-chain aliphatic alcohols, and fructooligosaccharides [26]. The plant's phytochemical composition suggests potential for treating UC and preventing its main complication, cancer [27]. However, there is currently no referenced preclinical research to support this use, so further pharmacological studies are necessary. Therefore, we propose to evaluate the anti-inflammatory activity of hydroalcoholic and butanolic extracts of *Agave brittoniana* subsp. *brachypus (*Trel.) in a model of acute experimental UC induced by acetic acid in rats.

2. RESULTS AND DISCUSSION

In the study, the aqueous suspension of the hydroalcoholic extract (HAE) was administered to the respective groups at doses of 150, 300 and 600 mg/kg bw, based on preclinical studies with similar natural preparations [28, 29] and other previous unpublished studies. The butanolic fraction (BF) was administered at doses of 25, 50, and 100 mg/kg of weight, based on previous anti-inflammatory evaluation studies conducted with this species [30]. The results were compared between the group treated with prednisolone (10 mg/kg) and the untreated group (with and without induction of UC by acetic acid).

2.1. Body Weight Variation

To analyse the variation in body weight at the end of the 48-hour study, we conducted a one-way ANOVA test. The WTC group, which did not receive any treatment, experienced a net loss in body weight. In the groups with acetic acid-induced colitis, a lower weight increase was observed compared to the healthy group ($p < 0.05$), except for the group treated with a 25 mg/kg dose of BF, which ended the study with a body weight similar to the healthy group.

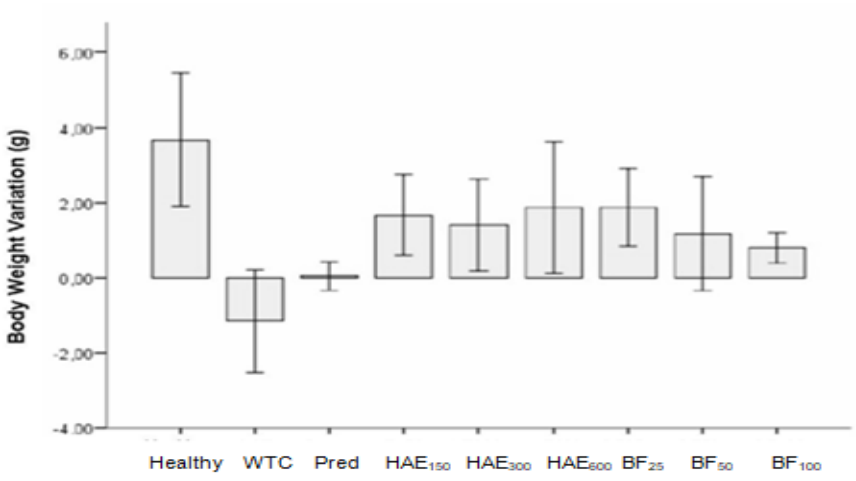

Figure 1. Body weight variation (BWV). The bars represent the limits with 95 % confidence. Healthy: Control Group, WTC: Without Treatment Control, Pred: Prednisolone group, HAE: Hidroalcoholic Extract, BF: Butanolic Fraction

The factors responsible for stimulating and maintaining colitis include enhanced vasopermeability, extended neutrophil infiltration, and elevated levels of inflammatory mediators in the intestinal mucosa [2]. In acetic acid-induced colitis, weight loss results from intestinal inflammation and subsequent water loss [32]. Ghobadi *et al*. [33] reported similar results to those obtained in the present investigation. They observed a significant decrease in body weight of acetic acid-induced colitis after 72 hours, even in treated groups.

2.2. Macroscopic characterization

To characterize the macroscopic features of the rat intestine, the macro Colonic Damage Index (CDI) was used to quantify the extent and severity of colonic damage. The mean IDC values for each group were compared to the controls using the non-parametric Mann Whitney test. (table 1).

Table 1. Macroscopic Colonic Damage Index.

GROUPS	CDI	p1	p2
HAE 150	8.33± 1.86	< 0.05	> 0.05
HAE 300	6.6 ± 2.70	< 0.05	> 0.05
HAE 600	3.40 ± 1.95	> 0.05	< 0.05
BF 25	5.33 ± 0.58	< 0.05	< 0.05
BF 50	2.00 ± 0.82	> 0.05	< 0.05
BF 100	5.5 ± 0.71	< 0.05	< 0.05
Pred	8.5 ± 3.00	< 0.05	> 0.05
WTC	9.33 ± 1.21	< 0.05	-
Healthy	0.17 ± 0.41	-	< 0.05

p1 vs Healthy group, p2 vs WTC group. CDI: Colonic Damage Index HAE: Hidroalcoholic Extract, BF: Butanolic Fraction. Pred: Prednisolone group. WTC: Without Treatment Control. Healthy: Control Group. The results are expressed as the Mean ± SD.

The analysis of the results indicates that the highest dose of HAE and the mean dose of BF have a significantly lower CDI than the WTC group, similar to the Healthy group. The doses of 25 and 100 mg/kg of BF reached a CDI significantly lower than the WTC group, although a CDI similar to the Healthy group was not achieved with these doses. The groups receiving 150 and 300 mg/kg HAE and prednisolone showed similar behaviour to the WTC group. ($p > 0.05$).

The WTC group showed a predominance of more than one area of ulceration or a large ulcer that caused graduations between 7 and 10. In the HAE group, smaller ulcers and damaged regions (graduations between 0 and 4) were observed with 600 mg/kg and BF 50 mg/kg. Graduations between 5 and 10 were observed in the groups of 150 and 300 mg/kg of the HAE, 25 and 100 mg/kg of BF, and prednisolone.

The figures presented in Figure 1 illustrate the observations made in the experimental groups.

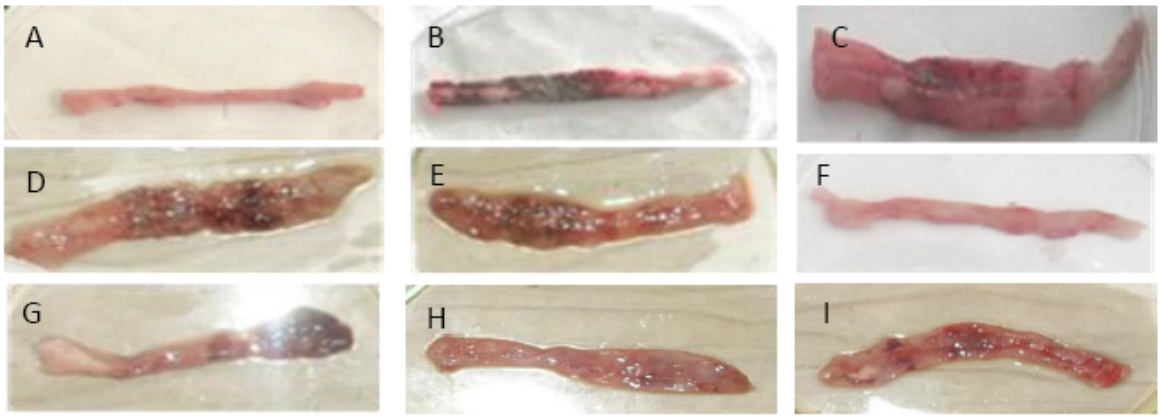

Figure 2. Colon Fragments: A) Healthy colon with normal mucosa, without erythema, inflammation or ulceration. B) WTC colon shows an extensive zone of ulceration with mucosal necrosis, graded at 10. C) Prednisolone colon exhibits areas of hemorrhage and tissue inflammation, graded at 4. D) HAE 150 mg/kg resulted in an extensive area of ulceration accompanied by inflammation, with a graduation of 7. E) HAE 300 mg/kg resulted in a zone of necrosis and ulceration accompanied by inflammation, with a graduation of 5. F) HAE 600 mg/kg resulted in a zone of bleeding without ulceration, with a graduation of 2. G) FB 25 mg/kg resulted in an area of necrosis and ulceration accompanied by inflammation, with a graduation of 5. H) FB 50 mg/kg resulted in a slightly thickened area with signs of hemorrhage, with a graduation of 2. I) FB 100 mg/kg resulted in an ulcer over 1 cm, with a graduation of 5.

Table 2 shows the percentages of macroscopic damage inhibition. The groups treated with agave exhibited higher inhibition percentages than the positive control, prednisolone, used in our study. The highest inhibition percentage was observed with doses of 600 mg/kg of HAE and 50 mg/kg of BF.

Table 2. Inhibition Percentage (IP) of macroscopic damage.

Groups	Pred	HAE 150	HAE300	HAE600	BF 25	BF50	BF100
IP	8.93	10.71	29.29	63.57	42.86	78.57	41.07

Pred: Prednisolone group, HAE: Hidroalcoholic Extract, BF: Butanolic Fraction.

The obtained results align with those of other authors who have noted that diluted acetic acid acts as a suitable local chemical irritant, causing lesions and ulcerations of the colonic tissue of rats [32]. Furthermore, several authors have established the efficacy of plants in ameliorating ulcerative damage caused by acetic acid. *Sorbus domestica* and *Momordica charantia* possess anti-inflammatory and antioxidant properties that inhibit inflammatory mediators in acute experimental colitis [34,35]. Mannasaheb *et al.* [36] demonstrated the protective effect of *Agave americana* against acetic acid-induced colitis in rats. The groups treated with agave showed an intact intestinal mucosa, along with regeneration of intestinal epithelial cells, providing a protective effect.

On the other hand, saponins from other species also have great antiulcer potencial. *Pulsatilla chinensis* saponins showed a high anti-inflammatory effect in the DSS model of ulcerative colitis [37, 38]. Some studies have reported the efficacy of Codonopsis steroidal saponins in various models of ulcerative colitis [39, 40].

Steroidal saponins and sapogenins are able to act directly on pro-inflammatory cytokines mainly through the Nf-κB, TLR4 and MAPKs pathways [41,42]. For instance, convallatoxin, can inhibit the expression of inflammatory factors by increasing the expression of PPARγ, thereby reducing colonic mucosal inflammation and improving experimental colitis [43].

Histological damage was assessed by estimating leukocyte infiltration, fibrosis, neutrophil marginalization to the submucosa, edema in the submucosa, epithelial necrosis, and epithelial ulceration. Table 3 shows the average results of each indicator according to Millar *et al*. [44].

Table 3. Microscopic colonic damage

Group	ELI	LILP	LIMM	LISM	LIMLP	SLI	MF	SMF	SMNM	SME	EN	EU
HAE150	1.5	0	0	2	1	0	0	0	0	0	0	0
HAE300	1	0	0	2	0	0	0	0	0	0	1	0
HAE600	0	0	0	0.25	0	0	0.12	0.12	0.12	0.12	0	0
BF25	1	0	0	1	1	0	0	0	0	0	0	0
BF50	1	0	2	0.67	0.67	0	0	0	0.33	0.67	0.67	0
BF100	1	0	0	1.6	0.4	0	0.2	0.2	0	1	0.75	0
Pred	1.8	2	0	2	0	0	0.6	0.4	1	1.6	1	1
WTC	2	2	2	2	2	0	2	1	1	2	2	1
Healthy	0	0	0	0	0	0	0	0	0	0	0	0

ELI: Epithelial Leukocyte Infiltration, LILP: Leukocyte Infiltration in the Lamina Propia, LIMM: Leukocyte Infiltration in the Muscle Mucose, LISM: Leukocyte Infiltration in the Submucosa, LIMLP: Leukocyte Infiltration in the Muscularis Lamina Propia SLI: Serous Leukocyte Infiltration, MF: Mucosal Fibrosis, SMF: Submucosal Fibrosis, SMNM: Submucosal Neutrophil Margination, SME: Submucosal Edema, EN: Epithelial Necrosis, EU: Epithelial Ulceration.

The microscopic analysis revealed that the WTC group exhibited tissue damage consistent with an inflammatory process, including severe leukocyte infiltration, fibrosis, and cases of necrosis and epithelial ulceration. In contrast, no microscopic damage was observed in the healthy control group. Similar to other UC models induced by TNBS, early-stage damage is characterized by discrete foci of necrosis and inflammation [45].

Most of the plant products tested at different doses reduced leukocyte infiltration in the lamina propria and muscle mucosa to 0, as well as epithelial ulceration, compared to the WTC group. Table 4 summarises the microscopic damage index values for each experimental group, determined according to the criteria of Millar *et al.* criteria [44].

Table 4. Microscopic colonic damage index.

GROUPS	CDI (MEDIA ± DS)	p1	p2
HAE 150	4.5 ± 2.12	< 0.05	< 0.05
HAE 300	4.00± 2.57	< 0.05	< 0.05
HAE 600	6.00 ± 2.27	< 0.05	< 0.05
BF 25	6.00 ± 0.93	< 0.05	< 0.05
BF 50	4.83 ± 2.6	< 0.05	< 0.05
BF 100	4.8 ± 0.45	< 0.05	< 0.05
Pred	11.00± 1.22	< 0.05	-
WTC	18.7 ± 0.55	-	< 0.05

p1 vs WTC: Without Treatment Control group, p2 vs Pred: Prednisolone group. HAE: Hidroalcoholic Extract, BF: Butanolic Fraction. Pred: Prednisolone group. WTC: Without Treatment Control. The results are expressed as the Mean ± SD.

The groups treated with *A. brittoniana* extract had significantly lower microscopic CDI levels compared to the WTC and prednisolone groups. Figure 2 displays colon fragments from the experimental groups and their corresponding microscopic findings.

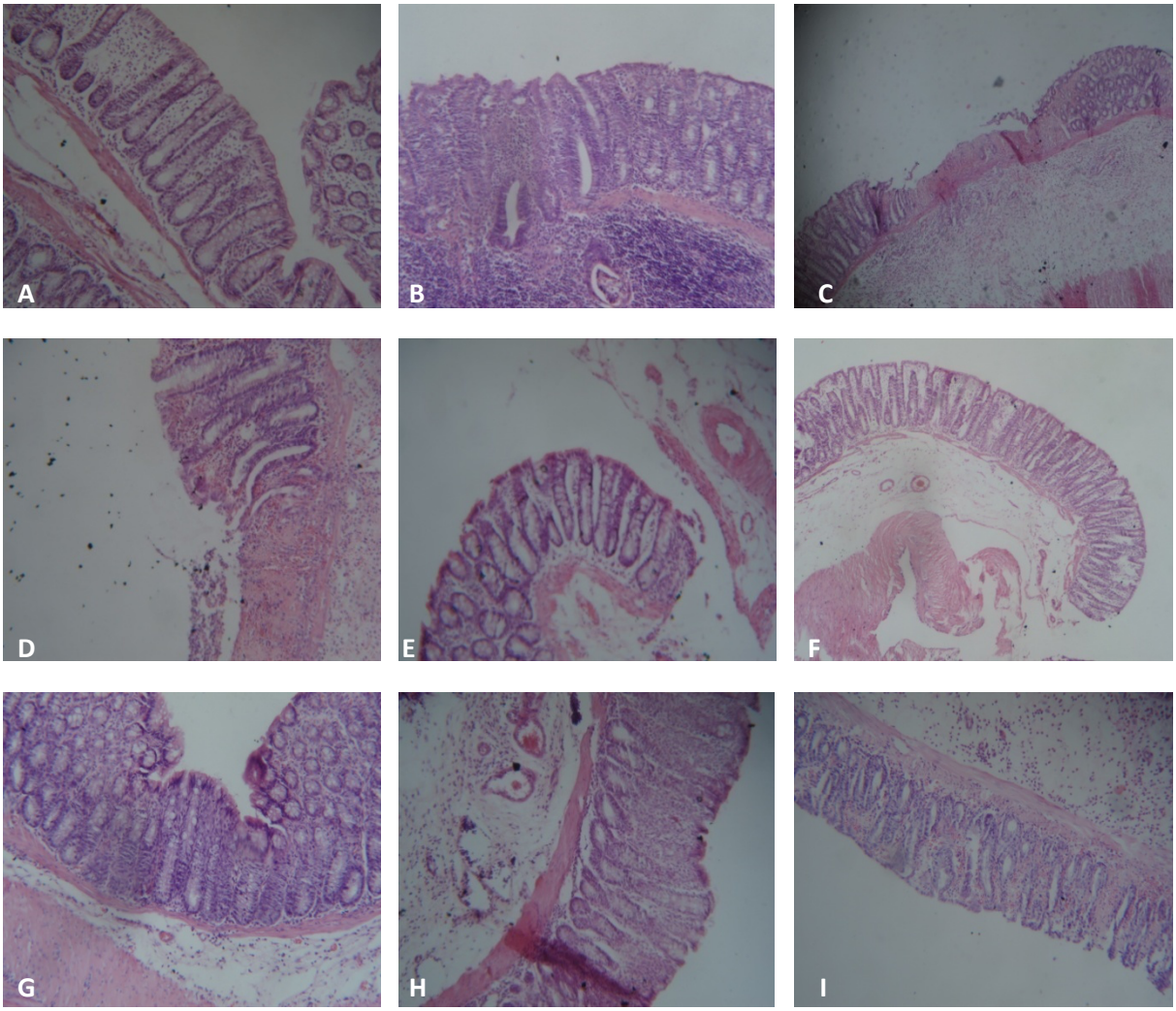

Figure 3. Images of 10x colon samples. A: Healthy, tissue is observed without damage. B: WTC group, cryptitis, microabscesses and dysplasia are observed. C: Prednisolone group. Fibrosis, ulceration and focal edema are observed. D) HAE 150 mg / kg: Leukocyte infiltration in submucosa and loss of epithelium. E) HAE 300 mg / kg. Leukocyte infiltration in submucosa and epithelial necrosis F) HAE 600 mg / kg: no tissue damage is observed. G) FB 25 mg / kg: Leukocyte infiltration in the submucosa and the muscular ones, with conserved epithelium. H) FB 50 mg / kg: Leukocyte infiltration in the submucosa and the muscularis propria and neutrophil margination I) FB 100 mg / kg: Leukocyte infiltration and edema in the submucosa.

UC is a condition that causes inflammation in the large intestine's mucosa, primarily affecting the colon's distal region (rectum-anus). The inflammatory process can cause the lesion to progressively extend continuously, altering the superficial layers of the intestinal mucosa and submucosa, resulting in a granular appearance. The lamina propia shows necrosis of the epithelium, edema, and infiltration of inflammatory cells, including neutrophils and eosinophils. [45, 46].

The literature reports that the WTC group exhibited clear signs of inflammation and necrosis in colonic tissue. Treatment with the plant reduced the damage, likely due to the anti-inflammatory activity of the saponins in the species and the healing effect of its plant fibre [19, 30, 47]. The steroidal saponins could modulate the action of pro-inflammatory cytokines and macrophages, thereby reducing inflammation of the colonic mucosa [41]. According to the role of intestinal microbiota, vegetable fibre may also contribute to the anti-inflammatory effect on colonic mucosa. Short-chain fatty acids resulting from intestinal microbiota metabolism have an anti-inflammatory effect by controlling the production of T helper cells, antibodies, and cytokines [48].

2.3. Determination of the myeloperoxidase (MPO) activity

The results of the MPO activity demonstrate the usefulness of this biomarker in evaluating the recovery or inhibition of tissue damage in UC. The graph below illustrates the behaviour of this parameter in each experimental group.

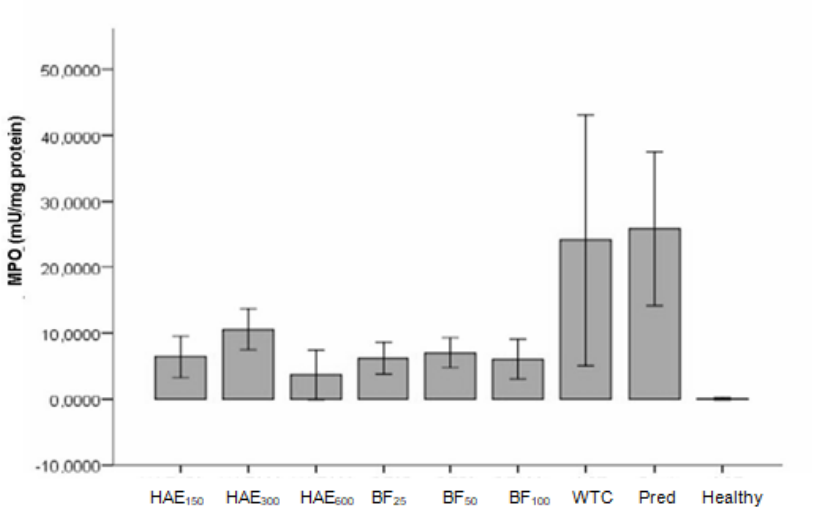

Figure 4. Colonic myeloperoxidase activity. The bars represent the means with the standard deviations.

A one-way ANOVA analysis was conducted to assess the activity of intestinal MPO, which revealed highly significant differences between the groups ($p < 0.05$). The parameter showed values higher than the healthy control in all groups exposed to the agent inducing colitis. These results are consistent with those of other authors who have found the sensitivity of MPO in different models of acute UC [49,50]. The Bonferroni post hoc test revealed that the MPO activity values of all agave groups were lower than the untreated colitis group. Additionally, the results indicated that the MPO activity in all agave groups was lower than that of the prednisolone group. The observed behaviour may be attributed to the steroidal saponins found in the extracts. Previous studies using saponin-rich extracts of *A. brittoniana* have shown significant anti-inflammatory effects in both acute and chronic inflammation models. [30, 47].

Table 5 displays the percentage of MPO activity inhibition in the treated groups compared to the untreated colitis group. All three doses of the agave products tested exhibited a higher percentage of inhibition than the prednisolone control group.

Table 5. Percentage of inhibition of the activity of the MPO

Groups	Pred.	HAE150	HAE300	HAE600	BF25	BF50	BF100
MPO	7.72	69.71	50.24	68.32	61.30	82.86	71.52

Pred: Prednisolone group. HAE: Hidroalcoholic Extract, BF: Butanolic Fraction.

MPO is a biomarker of oxidative damage and a leukocyte infiltration indicator in injured tissue. In an experimental model of UC, Yuan *et al*. [51] demonstrated that a preparation of Chinese medicinal plants with anti-inflammatory and antioxidant effects reduced plasma MPO levels and improved the inflammatory states of colonic mucosa.

All groups of agave demonstrated effectiveness in treating acetic acid-induced colitis, as evidenced by regression in most of the evaluated indicators. The best results, however, were achieved with doses of 600 mg/kg HAE and 50 mg/kg BF. The anti-inflammatory effect of the substance may be attributed to the presence of steroidal saponins and vegetable fibre, which may have a healing effect on the ulcerated colon.

3. CONCLUSION

As previously mentioned, the steroidal saponins from *A. brittoniana,* present in both the hydroalcoholic and butanolic extracts, may contribute to the protective effect on the intestinal mucosa. These metabolites have the potential to modulate inflammatory cytokines. On the other hand, vegetable fibres, which are also present in this species, have a cytoprotective effect due to their trophic role in the intestinal microbiota, preventing ulceration and cell damage. Both saponins and vegetable fibres have the potential to ameliorate the inflammatory state and the injury caused by acetic acid in this UC model. For the first time, the potential of this species in treating UC is demonstrated. Further studies are necessary to assess the contribution of metabolites and propose an anti-colitis pharmaceutical agent from this agave species.

4. MATERIALS AND METHODS

4.1. Plant material

The plant specimens were collected from the protected area of Cubanacán, which is adjacent to the city of Santa Clara. To identify the specimens, they were compared with samples from the herbarium of the Central University 'Marta Abreu' of Las Villas, identified under the serial number HPVC # 5445.

4.2. Preparation of extracts from *A. brittoniana* subsp. *brachypus*

The process of drying and grinding followed the previous work with this species. Subsequently, 300 g of the dried leaf powder underwent maceration with 1 L of 70% ethanol in the dark. This process was repeated every other day for seven days until the mixture was completely decolorized. The hydroalcoholic extract was obtained and then rotoevaporated using an IKA® RV 10 basic rotoevaporator at 40°C and steam pressure until a pasty mixture containing the crude metabolites was obtained. The resulting mixture was then dried completely in an oven at 40°C. The dried hydroalcoholic extract (10 g) was resuspended in water and placed in a separatory funnel. Saturated n-butanol was added and the mixture was left for 24 hours. The presence of saponins was confirmed using the foam test. The organic phase was then rotoevaporated until completely dry.

4.3. Experimental Design

The Animal Ethics Committee of the Experimental Toxicology Unit belonging to the Medical College of Villa Clara approved the experimental protocol, in accordance with the Principles of Laboratory Animal Care [52] and the Decree-Law N031 on Animal Welfare of the Republic of Cuba [53]. The rodents used in the experiment were purchased from the National Centre for Laboratory Animal Production. (Havana, Cuba).

Rats were randomly allocated into nine groups (n=6) including healthy group, WTC group, prednisolone-treated group (10 mg/kg; v.o), HAE-treated groups (150-300-600 mg/kg v.o) and BF- treated groups (25-50-100 mg/kg; v.o). Animals were fasted for 18 h and anaesthetised with ether, and a single intracolonic dose of 1 mL of 2.5% acetic acid (Unichem) was administered to induce UC [40]. Before the rectal application of the irritant, the area corresponding to 8.0 cm of rectum was washed with 0.9 % SSF, which coincides with the place of application. The acetic acid was removed with 0.9% SSF within 60 seconds of contact with the mucosa. To avoid any mechanical damage, a cannula was introduced using a hydrophilic lubricant (QUIMEFA) after instillation. All groups, including the WTC group, underwent the same procedure to eliminate bias due to the use of the moisturizing gel. Water was administered to the WTC and healthy groups. Only 0.9% SSF was administered to the healthy group, using the same route of administration to induce UC.

The substances were orally administered every 24 hours following instillation of acetic acid. Oral administration was carried out using an oesophageal tube at a volume of 5 mL/kg. The animals were weighed at the beginning of the test and before being slaughtered 48 hours after acetic

acid induction [54, 55]. After sacrifice, colonic tissue samples were taken for weighing, biochemical determinations, and macroscopic and microscopic analysis.

4.4. Macroscopic characterization

Following the sacrifice of the rats, a 10 cm sample of damaged colon was aseptically extracted from each animal. The samples were then cleaned with saline and placed on ice.

The extent and severity of colonic damage were assessed macroscopically by two observers using a scale of 0 to 10 proposed by Bell [56]. The macroscopic colonic damage index for the group was determined by calculating the mean value given by the observers for each animal.

4.5. Microscopic characterization

Colon samples measuring 0.5 cm in length were analysed from the area adjacent to the largest macroscopic damage. The colonic tissue was fixed in a buffered solution of formaldehyde (4 % in phosphate buffer, pH 7.2) for three days. Afterward, 3-5 µm thick, were cut using a microtome and stained with haematoxylin-eosin for evaluation under light microscopy. The sections were assessed by two pathologists based on the criteria established by Millar *et al.* [44].

The formula used to evaluate the percentage inhibition of macro- and micro-colonic damage was as follows: % Inhibition CD = (CDIc -CDIt)/CDIc * 100

CD: Colonic damage

CDIc: Colonic damage Index (macro o micro) control group

CDIt: Colonic damage Index (macro o micro) treated group

The study determined the colonic damage index for all parameters assessed in each case and calculated the mean per group.

4.6. Obtaining colonic homogenates

The colon fragments, which were previously weighed (100 mg) and crushed on ice, were homogenized using a Heidolph homogenizer in a solution of 0.5% w/v hexadecyltrimethylammonium bromide (HDTMA) in phosphate-buffered saline. The final solution was prepared at a ratio of 1:20 (p/v) and homogenized for 1 minute. The homogenate was sonicated using a Sonics Vibracell for 20 minutes and then underwent three freeze/thaw processes. Following the final thaw, the homogenate was centrifuged at 3000 rpm for 10 minutes at 4°C. The resulting supernatants were used to determine MPO activity.

4.7. Myeloperoxidase determination

Fragments of colon that corresponded to the area of greatest macroscopically observable damage were extracted. Each animal's 100 mg fragments were weighed and preserved in liquid nitrogen for the first 48 hours, and then at -40°C for processing during the first 96 hours of extraction.

To determine MPO levels, we utilized the o-dianisidine technique outlined by Graff [57]. This method involves the reduction of hydrogen peroxide by MPO, with the oxygen released in this reaction reacting with o-dianisidine to produce a coloured compound. The intensity of the colour is directly proportional to the MPO activity.

10 µL of the supernatant was mixed with 200 µL of o-dianisidine dichloroethane buffer, followed by the addition of 50 µL of 1% hydrogen peroxide. Each test was performed in duplicate and the mixture was incubated for 3 minutes at 37°C. The sample was centrifuged at 3000 rpm for 5 minutes. Enzyme activity was determined using the end-point method on a Photolab 6100vis spectrophotometer at 460 nm. One unit of MPO activity was defined as the amount of enzyme required to catalyse an increase in absorbance of 0.1 per minute at 460 nm at 25 °C. Specific MPO activity was expressed as mU/mg protein. Total proteins were determined using the Lowry method. [58].

4.8. Statistical analysis

The statistical package SPSS for Windows version 20.0 was used to process the data. The mean and standard deviation of each experimental group were determined. To test for differences between the experimental groups, a one-way ANOVA was applied after checking the normality of the distribution of the variables, followed by a Dunnet's test. Variables that did not follow a normal distribution were compared using the Kruskal-Wallis and Mann-Witney tests with a confidence interval of 95%.

Acknowledgements: This work was supported by National Program of Basic and Natural Sciences (PN223LH010-020) and Territorial Health Program (PT241VC001-001).

Author contributions: Concept – Y.G.M.; Design – Y.G.M, L.N.R.; Supervision – J.O.G.L.; Resources – Y.G.M.; Materials – D.S.M.; Data Collection and Processing – D.S.M., Y.G.M., M.L.G.G.; Analysis and Interpretation – Y.G.M., L.N.R., M.L.G.G; Literature Search – D.S.M., Y.G.M.; Writing – Y.G.M., D.S.M; Critical Reviews – L.N.R., J.O.G.L.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] Armuzzi A, Liguori G. Quality of life in patients with moderate to severe ulcerative colitis and the impact of treatment: a narrative review. Dig Liver Dis. 2021;53(7):803-808 . https://doi.org/10.1016/j.dld.2021.03.002
[2] Raine T, Bonovas S, Burisch J, Kucharzik T, Adamina M, Annese V, Bachmann O, Bettenworth D, Chaparro M, Czuber-Dochan W, Eder P, Ellul P, Fidalgo C, Fiorino G, Gionchetti P, Gisbert JP, Gordon H, Hedin C, Holubar S, Iacucci M, Karmiris K, Katsanos K, Kopylov U, Lakatos PL, Lytras T, Lyutakov I, Noor N, Pellino G, Piovani D, Savarino E, Selvaggi F, Verstockt B, Spinelli A, Panis Y, Doherty G. ECCO Guidelines on Therapeutics in Ulcerative Colitis: Medical Treatment. J Crohns Colitis. 2022;16(1):2-17. https://doi.org/10.1093/ecco-jcc/jjab178
[3] Thiede J, Eggli U. Agavaceae. In: Eggli U, Nyffeler R. (eds) Monocotyledons. Illustrated Handbook of Succulent Plants. 2020. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-56486-8_112
[4] Torres IG, Rendón FSJ, Blancas J, Moreno AIC. The genus Agave in agroforestry systems of Mexico. Bot. Sci. 2019; 97(3): 263-290. https://doi.org/10.17129/botsci.2202.
[5] Eguiarte LE, Jiménez Barrón OAB, Aguirre E, Scheinvar E, Gámez N, Gasca JP, Souza V. Evolutionary ecology of Agave: distribution patterns, phylogeny, and coevolution (an homage to Howard S. Gentry). Am J Bot. 2021; 108(2):216-235. https://doi.org/10.1002/ajb2.1609
[6] Alducin CM, Ruiz KM, Jiménez OB, Aguirre Planter EP, Gasca JP, Eguiarte LE, Medellin RA. Uses, knowledge and extinction risk faced by agave species in Mexico. Plants. 2023; 12: 124. https://doi.org/ 10.3390/plants12010124
[7] Eguiarte L, Souza V, Silva AM. Evolución de la familia Agavaceae: Filogenia, biología reproductiva y genética de poblaciones. Bot Sci. 2000; 66:131-150. https://doi.org/10.17129/botsci.1618
[8] POWO. Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. http://www.plantsoftheworldonline.org/ (accessed on consulted 7 February 2024).
[9] Davis S, Dohleman F, Long S. The global potential for Agave as a biofuel feedstock. Glob Chang Biol. 2011; 3(1): 68–78. https://doi.org/10.1111/j.1757-1707.2010.01077.x
[10] Callen EO. Food habits of some pre-Columbian Mexican Indians. Econ Bot. 1965; 19(4): 335-343.
[11] Casas A, Caballero J, Valiente-Banuet A. Use, management and domestication of columnar cacti in south-central Mexico: A historical perspective. J Ethnobiol. 1999; 19(1): 71-95. https://ethnobiology.org/sites/default/files/pdfs/JoE/19-1/Casas-etal.pdf
[12] Colunga PGM, May FP. Agave studies in Yucatan, Mexico. I. Past and present germplasm diversity and uses. Econ Bot. 1993: 312-327.
[13] Sánchez EA, Maiti RK, Wesche PE, Ramírez FB, Díaz AP, Singh VP. Contribution to medicinal uses and chemistry of some species of Agave: A review. Crop Res J. 2001;23: 375-381.
[14] Santos LZ, Leal, AMD, Cortes-Cehallos EC, Gutiérrez JAU. Agave (Agave spp.) and its traditional products as a source of bioactive compounds. Curr Bioact Compnds. 2012; 8(3): 218-231. https://doi.org/10.2174/157340712802762410
[15] Debnath M, Pandey M, Sharma R, Thakur RS, Lal P. Biotechnological intervention of *Agave sisalana*: a unique fibre yielding plant with medicinal property. J Med Plant Res. 2010; 4 (3): 177-187.
[16] Guevara, A, Vallejo, C. Medicinal potential of the genera Furcraea and Agave. Rev Cubana Plant Med. 2014; 19 (3): 248-263.
[17] Juang Y, Liang P. Biological and pharmacological effects of synthetic saponins. Molecules. 2020; 25(21):4974. https://doi.org/10.3390/molecules25214974
[18] Hassan H, Sule M, Musa A, Musa K, Abubakar M, Hassan A. Anti-inflammatory activity of crude saponin extracts from five nigerian medicinal plants. Afr J Tradit Complement Altern Med. 2012; 9:250-255. https://doi.org/10.4314/ajtcam.v9i2.10
[19] Hernández-Valle E, Herrera-Ruiz M, Salgado GR, Zamilpa A, Ocampo ML, Aparicio AJ, Tortorıello J, Jiménez-Ferrer E. Anti-inflammatory effect of 3-O-[(6′-O-Palmitoyl)-Dglucopyranosyl Sitosterol]), from *Agave angustifolia* on ear edema in mice. Molecules. 2014; 19: 15624–15637. https://doi.org/10.3390/molecules191015624
[20] Misra AK, Varma SK, Kumar R. Anti-inflammatory effect of an extract of *Agave americana* on experimental animals. Pharmacogn Res. 2018; 10: 104–108. https://doi.org/10.4103/pr.pr_64_17
[21] Mwale M, Masika PJ, Francis J. Anti-inflammatory and analgesic activities of the aqueous leaf extract of *Agave sisalana* in rats. Sci Res Essays. 2012; 7: 1477–1484. https://doi.org/10.5897/SRE11.660.
[22] Trelease W. Agave in the West lndies. Memoirs 11f the National Academy of Sciences. 1913; 11: 1-55
[23] de Zayas AÁ. Los agaves de las Antillas. Bot. Sci. 1995; (57):37-48. https://doi.org/10.17129/botsci.1475

[24] Macías FA, Guerra JO, Simonet AM, Nogueiras C. Characterization of the fraction components using 1D TOCSY and 1D ROESY experiments. Four new spirostane saponins from *Agave brittoniana* Trel. spp. *brachypus*. Magn Reson Chem. 2007; 45: 615-620. https://doi.org/10.1002/mrc.2012

[25] Macías FA, Guerra JO, Simonet AM, Pérez AJ, Nogueiras C. Characterization of three saponins from a fraction using 1D DOSY as a solvent signal suppression tool. Agabrittonosides E–F. Furostane saponins from *Agave brittoniana* Trel. spp. brachypus. Magn Reson Chem. 2010; 48: 350-355. https://doi.org/10.1002/mrc.2590

[26] Guerra JO, Meneses A, Simonet AM, Macías FA, Nogueiras C, Gómez A, Escario JA . Saponinas esteroidales de la planta *Agave brittoniana* (Agavaceae) con actividad contra el parásito *Trichomona vaginalis*. Rev Biol Trop. 2008; 56 (4): 1645-1652. https://doi.org/10.15517/rbt.v56i4.5751

[27] Niu W, Chen X, Xu R, Dong H, Yang F, Wang Y, Zhang Z, Ju J. Polysaccharides from natural resources exhibit great potential in the treatment of ulcerative colitis: A review. Carbohydr Polym. 2021; 254. https://doi.org/10.1016/j.carbpol.2020.117189

[28] Fernández P, Figueredo Y, Domínguez C, Fernández I, Sanabria M, González R, Echevarría M. Efecto antiinflamatorio del extracto acuoso liofilizado de *Ocimum tenuiflorum* L en ratas. Acta Farm. Bonaerense. 2004; 23(4): 492-497. http://sedici.unlp.edu.ar/handle/10915/6689

[29] Syamsul ES, Andani F, Soemarie YB. Analgesic activity study of ethanolic extract of *Callicarpa longifolia* Lamk. in mice. Majalah Obat Tradisional. 2016; 21(2): 99-103. http://dx.doi.org/10.22146/tradmedj.12824

[30] González YM, Mena YL Martín DM, Valido AD, Guerra JO, Nieto LR. *In vivo* anti-inflammatory effect of saponin-enriched fraction from *Agave brittoniana* Trel subspecie *brachypus*. Ars Pharm. 2020;61(4):231-237. http://doi.org/10.30827/ars.v61i4.15352

[31] Patıl MVK, Kandhare DA, Bhıse SD. Anti-inflammatory effect of *Daucus carota* root on experımental colitis in rats. Int J Pharm Pharm Sci. 2012; 4(1): 337-343.

[32] Sharon P, Stenson WF. Metabolism of arachidonic acid in acetic acid colitis in rats: similarity to human inflammatory bowel disease. Gastroenterol. 1985; 88: 55-63. https://doi.org/10.1016/S0016-5085(85)80132-

[33] Ghobadi A, Heydarian M, Bahrami G, Hosseinzadeh L, Shokoohinia Y, Farzaei M, Aghaei K. Evaluation of the therapeutic effect of *Achillea wilhelmsii* C. Koch aqueous extract in acetic-acid-induced ulcerative colitis in rat. J Rep Pharm Sci. 2021; 10(1): 29-34. https://doi.org/10.4103/jrptps.JRPTPS_4_20

[34] Akkol E, Dereli F, Taştan H, Sobarzo-Sánchez E, Khan H. Effect of *Sorbus domestica* and its active constituents in an experimental model of colitis rats induced by acetic acid. J Ethnopharmacol. 2020; 251:112521. https://doi.org/10.1016/j.jep.2019.112521.

[35] Özbeyli D, Ali Ş, Aykaç A, Terali K, Çilingir-Kaya Ö, Şenkardeş İ, Sener G. Therapeutic effects of *Momordica charantia* L. ethanolic extract on acetic acid-induced ulcerative colitis in rats. Eur J Biol. 2021; 80(2): 119-128. https://doi.org/10.26650/EurJBiol.2021.1016222

[36] Mannasaheb BA, Kulkarni PV, Sangreskopp MA, Savant C, Mohan A. Protective effect of *Agave americana* Linn. leaf extract in acetic acid-induced ulcerative colitis in rats. Ayu. 2015; 36: 101-106 http://doi.org/10.4103/0974-8520.168998

[37] Liu Y, Zhou M, Yang M, Jin C, Song Y, Chen J, Gao M, Ai Z, Su D. *Pulsatilla chinensis* saponins ameliorate inflammation and DSS-induced ulcerative colitis in rats by regulating the composition and diversity of intestinal flora. Front Cell Infect Microbiol. 2021; 11:728929. https://doi.org/10.3389/fcimb.2021.728929.

[38] Niu C, Hu X, Yuan Z, Xiao Y, Ji P, Wei YM, Hua YL. Pulsatilla decoction improves DSS-induced colitis via modulation of fecal-bacteria-related short-chain fatty acids and intestinal barrier integrity. J Ethnopharmacol. 2023; 300:115741. https://doi.org/10.1016/j.jep.2022.115741.

[39] Liu XF, Qiao J, Gao J, Chen ZJ, Liu X. [Protective effects of total saponins of Codonopsis on ulcerative colitis induced by TNBS in rats and its mechanism]. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 2021;37(4):397-401. Chinese. https://doi.org/10.12047/j.cjap.6051.2021.032.

[40] Chen X, Li A, Yang P, Wen J. *Sijunzi tang, Codonopsis pilosula* polysaccharide and *Saccharomyces boulardii* modulate the gut microbiome to alleviate Dss-induced colitis in mice. On J Complement Alt Med. 2018; 1(1). https://doi.org/OJCAM.MS.ID.000503.

[41] Passos FRS, Araújo-Filho HG, Monteiro BZ, Shanmugam S, de Souza-Araújo AA, Guedes da Silva JRA, Thangaraj P, Quintans LJ, Quintans JDSS. Anti-inflammatory and modulatory effects of steroidal saponins and sapogenins on cytokines: Review of pre-clinical research. Phytomedicine. 2022; 96: 153842-https://doi.org/10.1016/j.biopha.2021.153842

[42] Jafari R, Shayesteh S, Ala M, Yousefi-Manesh H, Rashidian, Hashemian S, Sorouri M, Dehpour AR. Dapsone ameliorates colitis through TLR4/NF-kB pathway in TNBS induced colitis model in rat. Arch Med Res. 2021; 52(6): 595-602. https://doi.org/10.1016/j.arcmed.2021.03.005.

[43] Li MY, Zhang ZH, Wang Z, Zuo HX, Wang JY, Xing Y, Ma J, Jin CH, X GH, Piao LX, Jin X. Convallatoxin protects against dextran sulfate sodium-induced experimental colitis in mice by inhibiting NF-κB signaling through activation of PPARγ. Pharmacol Res. 2019; 147: 104355. https://doi.org/10.1016/j.phrs.2019.104355

[44] Millar A, Rampton D, Chander C, Claxson A, Blades S, Coumbe A, Panetta J, Morris CJ, Blake DR. Evaluating the antioxidant potential of new treatments for inflammatory bowel disease using a rat model of colitis. Gut. 1996;39(3):407-415. http://dx.doi.org/10.1136/gut.39.3.407

[45] Gong Y, Niu W, Tang Y, Zhang Q, Liu S, Liu X, Wang X, Xue Y. Aggravated mucosal and immune damage in a mouse model of ulcerative colitis with stress. Exp Ther Med. 2019; 17(3):2341-2348. https://doi.org/10.3892/etm.2019.7162.

[46] Bahrami G, Malekshahi H, Miraghaee S, Madani H, Babaei A, Mohammadi B, Hatami R. Protective and therapeutic effects of *Aloe vera* gel on ulcerative colitis induced by acetic acid in rats. Clin Nutr. 2020; 9(3):223-234. https://doi.org/10.7762/cnr.2020.9.3.223.

[47] Resino-Ruiz D, González-Madariaga Y, Nieto L, Mena Y, Guerra JO, Vázquez AV, Díaz, Valido AD, Torrens F, Castillo-Garit JA. Anti-inflammatory activity: *in silico* and *in vivo* of sapogenins present in *Agave brittoniana* subsp. *brachypus* (Trel.). Antiinflamm Antiallergy Agents Med Chem. 2023; 22 (1):42-48. https://doi.org/10.2174/1871523022666230419103027

[48] Ziegler TR, Evans ME, Fernández- Estívariz C, Jones DP. Trophic and cytoprotective nutrition for intestinal adaptation, mucosal repair and barrier function. Ann Rev Nutr. 2003; 23(1):229-261. https://doi.org/10.17226/5140

[49] Dejban P, Sahraei M, Chamanara M, Dehpour A, Rashidian A. Anti-inflammatory effect of amitriptyline in a rat model of acetic acid-induced colitis: The involvement of the TLR4/NF-kB signaling pathway. Fundam Clin Pharmacol. 2021; 35(5):843-851. https://doi.org/10.1111/fcp.12642.

[50] Chami B, Ahmad G, Schroder A, San-Gabriel P, Witting P. The role of myeloperoxidase and neutrophil extracellular traps in the pathogenesis of inflammatory bowel disease. Gastrointest Endosc Clin N Am. 2021; 160(3). https://doi.org/10.1053/j.gastro.2021.01.044.

[51] Yuang Z, Yang L, Zhang X, Ji P, Hua Y, Yamming W. Huang-lian-jie-du decoction ameliorates acute ulcerative colitis in mice via regulating NF-kB and Nrf2 signaling pathways and enhancing intestinal barrier function. Front Pharmacol. 2019;10:1354-1354. https://doi.org/10.3389/fphar.2019.01354

[52] National Research Council (US). Institute for Laboratory Animal Research. Guide For the care and use of laboratory animals. Washington DC. National Academis Press (US); 1996. https://doi.org/17226/5140

[53] Decreto-Ley No. 31 de Bienestar Animal. Publicado en la Gaceta Oficial de la República de Cuba. 2021:37. https://www.mined.gob.cu/wpcontent/uploads/2022/01/goc-2021-ex25-.pdf

[54] Shahriarirad R, Erfani A, Nekouei F, Seifbehzad S, Hosseinzadeh M, Sarkari B, Tanideh N, Koohi-Hosseinabadi O, Nassour N, Ashkani-Esfahani *S*. Protective effect of *Melissa officinalis* against acetic-acid induced ulcerative colitis in rat models: an experimental study. Ann Gastroenterol. 2023;36(6):654-660. https://doi.org/10.20524/aog.2023.0836

[55] Hagar HH, El Medany A, El Eter E, Arafa M. Ameliorative effect of pyrrolidinedithiocarbamate on acetic acid-induced colitis in rats. Eur J Pharmacol. 2007; 554(1):69-77. https://doi.org/10.1016/j.ejphar.2006.09.066

[56] Bell C, Gall D, Wallace J. Disruption of colonic electrolyte transport in experimental colitis. Am J Physiol Gastrointest Liver Physiol. 1995; 268(4 Pt 1):G622-630.https://doi.org/10.1152/ajpgi.1995.268.4.G622

[57] Graff G, Gamache D, Brady M, Spellman J, Yanni J. Improved myeloperoxidase assay for quantitation of neutrophil influx in a rat model of endotoxin-induced uveitis. J Pharmacol Toxicol Methods. 1998 39(3):169-178. https://doi.org/10.1016/S1056-8719(98)00023-9

[58] Kielkopf C, Bauer W, Urbatsch I. Methods for measuring the concentrations of proteins. Cold Spring Harb. Protoc. 2020; 2020(4). https://doi.org/10.1101/pdb.top102277.