Effectiveness of Propolis on Experimental Colitis Model in Rats

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Abstract

Aim: This study investigated the therapeutic potential of propolis in an experimental colitis model induced by acetic acid in female Wistar albino rats.

Methods: Thirty rats were divided into five groups: a control group and four experimental groups. Colitis was induced in the second, third, and fifth groups by rectal administration of 1 ml of 4% acetic acid. The third group received rectal propolis solution (50 mg/ml), while the fourth group was given only rectal propolis solution. The fifth group received 1 ml of olive oil rectally after the onset of colitis. Stool consistency and weight loss were monitored, and colon tissue samples were collected for microscopic and macroscopic evaluation. The levels of MDA, MPO, and caspase-3 in tissue, as well as TNF-α and IL-10 levels in blood samples, were examined.

Results: The group administered propolis showed a significant decrease in microscopic and macroscopic scores compared to the other experimental groups. The levels of MDA, MPO, and caspase-3 in the tissue, as well as TNF-α and IL-10 levels in blood samples, were significantly decreased in the propolis group compared to the other experimental groups. Weight loss and stool consistency also showed improvement in the propolis group compared to the other experimental groups.

Conclusions: Propolis may have therapeutic effects in experimental colitis induced by acetic acid. The decrease in oxidative damage and inflammation seen in the propolis group indicates that it may be a useful therapeutic agent for colitis treatment.

Keywords: *Acetic acids, colitis, ulcerative, rats, wistar, bee products, olive oil*

1. Introduction

The worldwide incidence and prevalence of inflammatory bowel diseases (IBD) is on the rise. [1] Although the etiology of IBD remains largely unknown, it is believed to result from an intricate interplay between genetic, environmental, microbial factors, and immune respons. [2, 3] The pathogenesis of IBD involves an uncontrolled immune system that interacts with the intestinal flora in genetically predisposed individuals, leading to an inflammatory response that primarily affects the digestive system. [4, 5] The main objective of medical intervention is to mitigate the extent of inflammation and maintain clinical remission.

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Various pharmacological agents, such as 5-aminosalicylate (5-ASA), corticosteroids, thiopurines, methotrexate, calcineurin inhibitors, infliximab, and adalimumab, are employed for treating IBD. [6]

Rectal administration of acetic acid (4-10%) via a feeding catheter has been demonstrated to induce acute colitis in rats, mice, rabbits, and guinea pigs. [7] This type of colitis is characterized by necrosis and edema in the mucosal epithelium, with later stages exhibiting inflammation in both the mucosal and submucosal layers. Inflammatory cells and mediators lead to severe tissue damage. [8]

Propolis is a resinous substance produced by bees that combines extracts from plant buds and exudates with bee enzymes, pollen, and wax. [9]Its known properties include antiseptic, antimicrobial, anti-inflammatory, antitumor, immunomodulatory, and antioxidant effects. [10]

Experimental models of colitis play a critical role in preclinical research for developing effective treatments for inflammatory bowel diseases. Various therapeutic agents have been evaluated using diverse experimental colitis models.

The primary objective of this study is to investigate the efficacy of propolis in acetic acid-induced colitis in rats, as reported in the existing literature.

2. Materials and methods

This study was conducted in accordance with ethical guidelines and received approval from the ethics committee of Çukurova University Health Sciences Experimental Application and Research Center (SABIDAM), under protocol number TTU-2022-14631 and decision number 1 of the Animal Experiments Local Ethics Committee of Çukurova University Faculty of Medicine, dated 20.01.2022.

Thirty female Wistar Albino rats with an average weight of 200-260 grams were obtained from Çukurova University Health Sciences Experimental Application and Research Center and used in the experiments. The rats were housed individually in cages with a 12-hour light-dark cycle, and were provided with tap water and standard pellet feed at room temperature of approximately 22°C throughout the study.

2.1. Induction of colitis:

The experimental colitis was induced by using a 4% solution of acetic acid in this study. The administration of 1 cc of the acetic acid solution was performed by inserting the 6 F polyurethane catheter approximately 6-8 cm into the rectal route. To prevent the backflow of the administered substance, the rats were kept in the Trendelenburg position for 30 seconds. Subsequently, each rat was placed in its individual cage.

Groups:

Groups of rats were assigned as follows (Table 1):

Group 1: *Control group, no intervention, sacrificed after 120 hours.*

Group 2: *Acetic acid group, received 1 cc of 4% acetic acid intrarectally at the start of the experiment, sacrificed after 120 hours.*

Group 3: *Acetic acid + Propolis group, received 1 cc of 4% acetic acid intrarectally at the start of the experiment, then 1 cc of 5% propolis solution rectally at 4, 24, 48, and 72 hours, sacrificed after 120 hours.*

Group 4: *Propolis group, no intervention at the start of the experiment, then received 1 cc of propolis solution rectally at 4, 24, 48, and 72 hours, sacrificed after 120 hours.*

Group 5: *Acetic acid + Olive oil group, received 1 cc of 4% acetic acid intrarectally at the start of the experiment, then 1 cc of extra virgin olive oil rectally at 4, 24, 48, and 72 hours, sacrificed after 120 hours.*

Before starting the experiment, all rats were weighed, and their weights were recorded. The rats were placed in individual cages throughout the study.

The rats were sedated with a combination of 10 mg/kg xylazine hydrochloride (XYLAZIN BIO ®, 2%, Bioveta PLC Ivanovice na Hane-Czech Republic) and 90 mg/kg ketamine (Ketasol ®, Richter Pharma AG, Wels-Austria) given by intraperitoneal injection. Blood samples were taken by intracardiac puncture (2 cc) and collected in EDTA tubes. A laparotomy was performed with a Y-shaped incision to remove the left colon and rectum. The consistency of the stool sample from the rectum and left colon was checked, and the removed tissue was washed with +4 celcius degrees of saline solution. Half of the tissue was fixed in a formaldehyde solution in a sterile container, and the other half was placed in an Eppendorf tube and stored in liquid nitrogen solution. The Eppendorf tube was then stored in a freezer at -80 degrees Celsius.

2.2. Preparation of Propolis

The solution was prepared by taking 10 grams of pure and powdered propolis sample produced in the Black Sea Region and adding it to 200 milliliters of olive oil, as described by Krell (1996). The solution was gently heated at no more than 50 degrees Celsius for about 10 minutes in a hot water bath with constant stirring. After filtering, it was taken into amber-colored glass bottles and stored at +4 degrees in the refrigerator. [11]

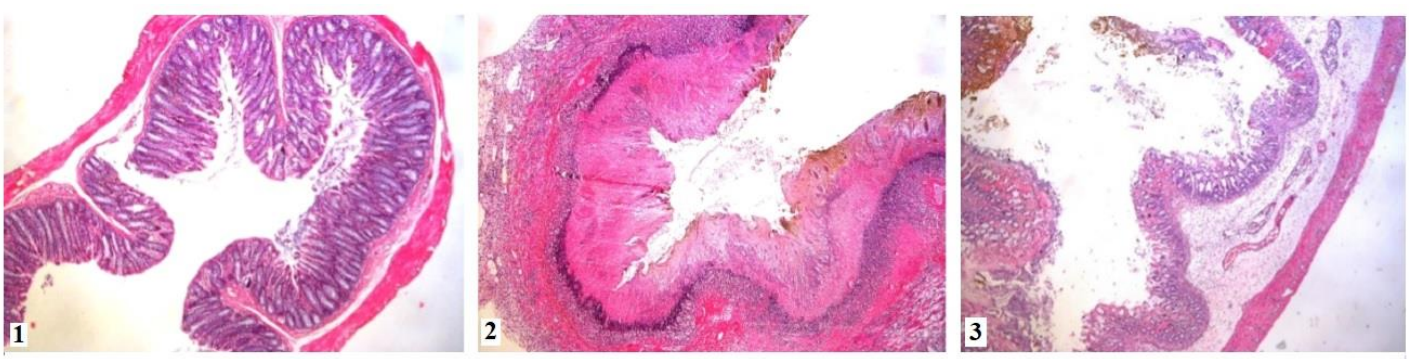
Before anesthesia was given to the rats in group 3 and group 4, they were administered 250mg/kg/day of the propolis solution rectally at the 4th, 24th, 48th, and 72nd hours.

2.3. Histopathological and Immunohistochemical Evaluation

The colon tissue was evaluated macroscopically and microscopically using scoring systems developed by Wallace and colleagues and Gaudio and colleagues, respectively. (Figures 1-5) Weight loss was assessed at the beginning and end of the study, and blood samples were collected for analysis of TNF-α, MPO, MDA, and IL-10 levels using ELISA kits and a SunRed ELISA microplate reader and washer. Absorbance was measured at 450 nm.

Figure 1, 2, 3

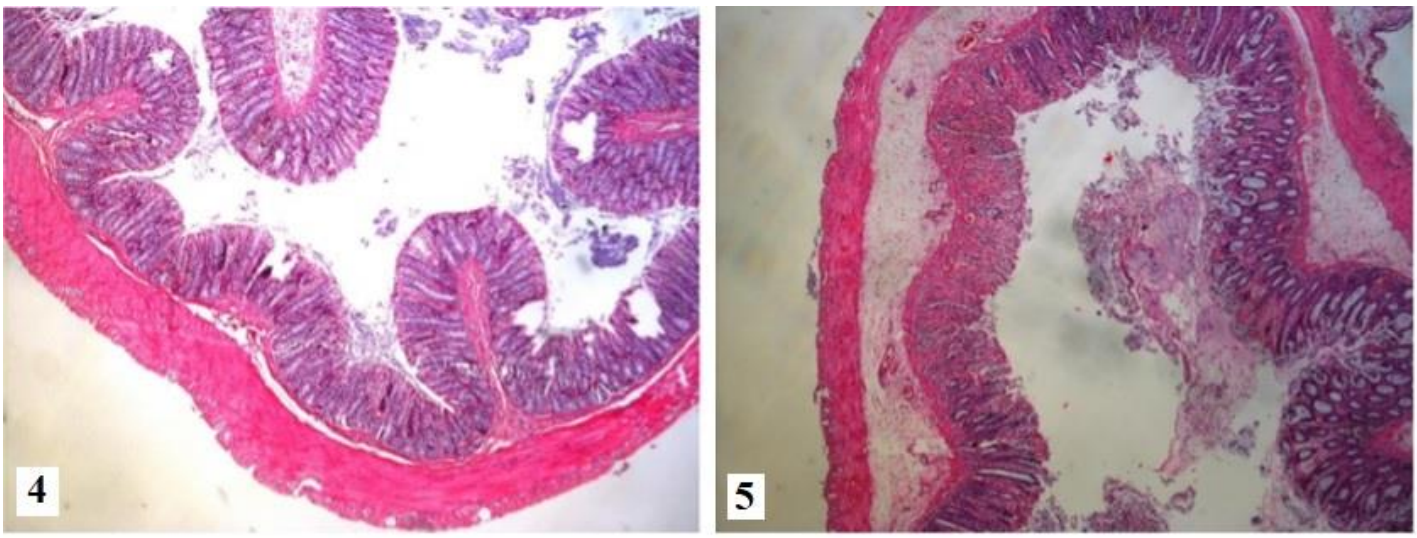
Microscopic view of control group, microscopic view of acetic acid group, microscopic view of acetic acid+propolis group



1. Microscopic view of control group [Control group rat colon histological image. A normal appearance is observed in the colonic mucosa. (40X magnification, H&E staining), 2. Microscopic view of acetic acid group [Acetic acid group rat colon histological image. Ulceration in the intestinal tissue, necrosis in the epithelium, acute and chronic inflammatory cells, submucosal edema, and fibrin deposition in the vessel walls are observed. (40X magnification, H&E staining)], 3. Microscopic view of acetic acid+propolis group [Mucosal damage is minimal, edema and inflammatory cells are observed in the submucosa (40X magnification, H&E staining)]

Figure 4, 5

Microscopic view of control group, microscopic view of acetic acid group, microscopic view of acetic acid+propolis group



4. Microscopic view of propolis group [Propolis group rat colon histological image. A normal appearance is observed in the colonic mucosa. (40X magnification, H&E staining)], 5. Microscopic view of acetic acid+olive oil group [Focal superficial ulceration, submucosal edema and mild inflammatory cells in the mucosa are observed. (40X magnification, H&E staining)]

2.4. Statistical analysis

Categorical data were presented as frequencies and percentages, while numerical data were expressed as mean and standard deviation (or median and range if necessary). The normal distribution assumption of the numerical data was checked by using the Shapiro Wilk test. One-Way Analysis of Variance was applied to compare the numerical data of more than two groups, provided that the assumptions were met. If the assumptions were not met, the Kruskal Wallis test was used instead. In cases where significant differences were detected among the groups, pairwise comparisons were performed using Bonferroni or Games & Howell tests, depending on the homogeneity of the variances. If the variances were not homogeneous, the Mann Whitney U test with Bonferroni correction was used. Statistical analysis was conducted using IBM SPSS Statistics Version 20.0, and the level of statistical significance was set at 0.05 for all tests.

3. Results

The study involved five groups, each consisting of six rats, with one rat in the acetic acid+olive oil group having died. The collected data, which included weight loss, stool consistency, macroscopic and microscopic scores, are displayed in Table 2.

Pairwise comparisons of weight loss scores revealed a significant difference between the acetic acid group and the propolis group ($p<0.05$), as well as between the propolis group and the acetic acid + olive oil group ($p<0.05$). Similarly, significant differences were found in stool consistency scores between the acetic acid group and the propolis and acetic acid + propolis groups ($p<0.05$), and between the propolis group and the acetic acid + olive oil group ($p<0.05$).

Table 1

Study groups

Groups	Protocol					
Group 1 (n=6)	-	-	-	-	-	Sacrification
Group 2 (n=6)	Acetic acid	-	-	-	-	Sacrification
Group 3 (n=6)	Acetid acid	Propolis	Propolis	Propolis	Propolis	Sacrification
Group 4 (n=6)	-	Propolis	Propolis	Propolis	Propolis	Sacrification
Group 5 (n=6)	Acetic acid	Olive Oil	Olive Oil	Olive Oil	Olive Oil	Sacrification
Hours	0	4	24	48	72	120

Microscopic scores differed significantly between the control group and the acetic acid group, and between the acetic acid and propolis groups ($p<0.05$). Furthermore, significant differences were detected between the acetic acid and acetic acid + olive oil groups in comparison to the control group, as well as between the acetic acid group and the propolis group ($p<0.05$) (Table 3).

Caspase-3 values differed significantly between the control group and the acetic acid group, between the acetic acid and propolis groups, and between the propolis group and the acetic acid + olive oil group ($p<0.005$). For MPO values, significant differences were found between the control group and the acetic acid, acetic acid + propolis, and acetic acid + olive oil groups ($p<0.05$).

Table 2

Weight Loss, stool consistency, macroscopic and microscopic scores by groups

Subjects	Groups	Weight Loss Score	Stool consistency	Macroscopic score	Microscopic score
1	CONTROL (K1)	0	1	0	0
2	CONTROL (K2)	0	1	0	0
3	CONTROL (K3)	1	1	0	1
4	CONTROL (K4)	0	1	0	0
5	CONTROL (K5)	0	1	0	1
6	CONTROL (K6)	0	2	0	1
7	ACETIC ACID (AA1)	4	3	6	18
8	ACETIC ACID (AA2)	3	3	7	17
9	ACETIC ACID (AA3)	3	3	6	18
10	ACETIC ACID (AA4)	2	2	7	18
11	ACETIC ACID (AA5)	3	3	6	20
12	ACETIC ACID (AA6)	4	3	5	17
13	ACETIC ACID + PROPOLIS (AA+PP1)	1	1	3	12
14	ACETIC ACID + PROPOLIS (AA+PP2)	1	2	2	8
15	ACETIC ACID + PROPOLIS (AA+PP3)	3	1	2	12
16	ACETIC ACID + PROPOLIS (AA+PP4)	3	1	3	12
17	ACETIC ACID + PROPOLIS (AA+PP5)	2	1	1	8
18	ACETIC ACID + PROPOLIS (AA+PP6)	2	2	3	10
19	PROPOLIS (PP1)	0	1	0	0
20	PROPOLIS (PP2)	0	1	0	0
21	PROPOLIS (PP3)	0	1	1	0
22	PROPOLIS (PP4)	1	1	0	2
23	PROPOLIS (PP5)	0	1	0	1
24	PROPOLIS (PP6)	1	2	0	0
25	ACETIC ACID + OLIVE OIL (AA+OO1)	3	2	5	14
26	ACETIC ACID + OLIVE OIL (AA+OO2)	2	2	4	12
27	ACETIC ACID + OLIVE OIL (AA+OO3)	2	1	2	8
28	ACETIC ACID + OLIVE OIL (AA+OO4)	3	2	4	14
29	ACETIC ACID + OLIVE OIL (AA+OO5)	4	3	3	13

A significant difference was also observed between the acetic acid + propolis group and the propolis group ($p<0.05$). Comparisons of TNF-α values between groups revealed significant differences between the control group and the acetic acid, acetic acid + propolis, propolis, and acetic acid + olive oil groups ($p<0.05$), as well as between the acetic acid + propolis group and the propolis group ($p<0.05$) (Table 3).

4. Discussion

The primary aim of therapeutic interventions in experimental models of colitis is to mitigate inflammation and minimize tissue necrosis. To evaluate the severity of inflammation and tissue damage, various parameters have been employed, including macroscopic and microscopic examinations, stool analyses, and more quantitative and objective biochemical and immunohistochemical measurements. Numerous bioactive compounds have been investigated for their protective effects against colitis via oral and rectal administration. The latter is considered safer due to the reduced toxicity and side effects of the drug. Oruc et al. (2008) investigated the potential efficacy of leflunomide in treating experimental colitis induced by acetic acid in rats. Their study demonstrated that intragastric administration of leflunomide significantly decreased the severity of colitis by reducing MDA levels. [12]

Propolis has been the subject of many experimental investigations exploring its positive effects. A systematic review conducted by Ruiz-Hurtado et al. (2021) examined the effects of orally administered propolis on gastric ulcer induced by non-steroidal anti-inflammatory drugs (NSAIDs) in the gastrointestinal system. The review analyzed studies conducted between 2000-2021 and demonstrated that propolis can be effective in treating NSAID-induced gastric ulcers. This effect is attributed to its antioxidative, anti-inflammatory, and cytoprotective properties, which inhibit gastric acid secretion

and pro-inflammatory cytokine release. [13]

Table 3

Statistical analysis of clinical, biochemical and immunohistochemical parameters

	Groups					p
	Control	Acetic acid	Acetic acid + propolis	Propolis	Acetic acid + olive oil	
Weight Loss Score	0.0(0.0-1.0)[a,d]	3.0(2.0-4.0)[c]	2.0(1.0-3.0)	0.0(0.0-1.0)[d]	3.0(2.0-4.0)	<0.001
Stool Consistency Score	1.0(1.0-2.0)[a]	3.0(2.0-3.0)[b,c]	1.0(1.0-2.0)	1.0(1.0-2.0)	2.0(1.0-3.0)	0.001
Macroscopic Score	0.0(0.0-0.0)[a]	6.0(5.0-7.0)[c]	2.5(1.0-3.0)	0.0(0.0-1.0)	4.0(2.0-5.0)	<0.001
Microscopic Score	0.5(0.0-1.0)[a]	18.0(17.0-20.0)[c]	11.0(8.0-12.0)	0.0(0.0-2.0)	13.0(8.0-14.0)	<0.001
Weight change	0.0(-6.0;0.0)[a,d]	-42.0(-52.0-20.0)[c]	-17.0(-40.0-4.0)	-1.0(-4.0;0.0)	-28.0(-42.0-16.0)	<0.001
Weight loss(%)	0.0(0.0;2.0)[a,d]	17.0(7.0;20.0)[c]	7.0(1.0;17.0)	0.0(0.0;1.0)	11.0(6.0;20.0)	<0.001
Caspase-3	7.5(5.0;20.0)[a]	45.0(40.0;60.0)[c]	20.0(10.0;30.0)	5.0(5.0;10.0)[d]	40.0(20.0;40.0)	<0.001
MPO	214.2±8.7[a,b,d]	271.0±11.3[b,c]	239.8±6.7[c]	212.4±21.2[d]	248.3±6.1	<0.001
MDA	24.9±3.1[a,b,d]	43.9±2.3[b,c,d]	31.4±1.9[c]	25.3±3.2[d]	35.0±3.0	<0.001
IL_10	229.5±14.8	198.6±73.0	197.0±46.3	189.8±26.8	183.6±35.1	0.465
TNF-α	177.1±16.3[a,b,c,d]	279.4±13.3[b,c,d]	233.8±13.1[c]	207.4±5.0	237.4±19.7	<0.001

Data are summarized as mean±standard deviation or median(min;max). ap<0.05 compared to acetic acid, bp<0.05 compared to acetic acid+propolis, cp<0.05 compared to propolis, dp<0.05 compared to acetic acid+olive oil.

Propolis has been suggested to possess anti-inflammatory properties in the context of colitis, possibly mediated through its impact on the gut microbiota. Krocko et al.(2012) found that the addition of propolis and bee pollen to chicken feed resulted in a decrease in the colonization of enterobacteria in chicken crops, but had no effect on lactobacilli. [14] Similarly, Wang et al. (2018) demonstrated that propolis administration reduced colitis severity, colonic apoptosis, and significantly reduced the colonization of bacterioides in the intestines. [15]

In experimental colitis models, propolis has shown a general anti-inflammatory and cytoprotective effect. One study by Khan et al. (2018) using a DSS-induced colitis model suggested that intraperitoneal administration of the active component of propolis, caffeic acid phenyl ester (CAPE), has a protective effect against colitis. However, the study also found that the level of anti-inflammatory cytokine IL-10 was lower in the CAPE-treated group compared to the colitis group, which was attributed to the suppressive effect of CAPE on IL-10. [16] In our study, we observed weight loss in the acetic acid group compared to the control group, and a significant difference in weight loss between the acetic acid group and the acetic acid+propolis group. Additionally, MPO and TNF-α values were significantly higher in the acetic acid group than in the acetic acid+propolis group, which supports the anti-inflammatory activity of propolis. However, we did not observe a statistically significant difference in IL-10 values between the acetic acid group and the acetic acid+propolis group.

Gonçalves et al. (2013) conducted a study on a TNBS-induced colitis model using rectal propolis and mesalazine in 50 rats. They evaluated colitis activity by examining stool consistency score, macroscopic score, microscopic score, and MPO activity using histological studies. In their study, colitis was induced with 20 mg of TNBS, and treatment was initiated 48 hours after colitis was induced using 0.8 ml of 8% propolis extract or mesalazine solution for 5 or 12 days. [17] The study showed that delaying the initiation of treatment led to the development of inflammation. The results of the study showed that there was no significant difference between the acetic acid+propolis group and the acetic acid group in terms of stool score, macroscopic score, microscopic score, and MPO values. In our study, on the other hand, we found a significant difference between the two groups in terms of stool consistency score and MPO, but no significant difference was found in microscopic and macroscopic scores. The difference in the results of the two studies may be due to the late onset of treatment in the other study.

According to a study conducted by Aslan et al. (2007), a distal colitis model induced with acetic acid was used to divide 40 mice into 5 groups. The control group constituted the first group, while the second group comprised the colitis group. The third and fourth groups consisted of the colitis + example enema group and the colitis + intragastric propolis group, respectively. The fifth group was designated as the colitis + mesalamine enema + intragastric propolis group. After sacrifice, the 8 cm distal colon was examined both histopathologically and biochemically. Histologically, less tissue damage was observed in the colitis + propolis group compared to both the colitis + mesalamine group and the colitis group. In the biochemical analysis, the colitis group exhibited significantly higher MDA values than the colitis + propolis group, although no significant difference was observed in the MPO values. [18] Our study observed statistically significant differences in both MDA and MPO values be-

tween the acetic acid and acetic acid + propolis groups. This discrepancy between the two studies may be attributed to the different route of propolis administration.

Activation of apoptotic pathways indicated by up-regulation of caspase-3 is considered a marker of colitis severity. [19, 20] Murad et al. (2022) investigated the effects of active olmesartan medoxomil on a TNBS-induced colitis rat model and assessed colitis activity score, MPO, TNF-α, IL-6, MDA, GSH, as well as E-cadherin, caspase 3, and matrix metalloproteinase-9 (MMP-9) expression in colon segments using immunohistochemistry. Olmesartan treatment led to significantly reduced MPO activity, TNF-α, and MDA levels compared to the colitis group. [21] In our study, we found that MPO activity, TNF-α activity, and MDA levels were significantly lower in the acetic acid+propolis group compared to the acetic acid group. However, while olmesartan treatment resulted in down-regulation of caspase-3 levels and prevented apoptotic pathway activation, the acetic acid + propolis group showed lower caspase-3 levels compared to the acetic acid group, with no significant difference between the two groups. This suggests that propolis' anti-apoptotic effect may be more limited than its anti-inflammatory effect.

There were significant differences between the acetic acid group and the control group in terms of various clinical, biochemical and immunohistochemical parameters including weight loss, stool score, macroscopic and microscopic scores, weight change and percent weight loss. These differences indicated the development of colitis in the acetic acid group, as demonstrated by elevated levels of Caspase-3, MPO, MDA and TNF-α.

In comparison, the propolis group showed no significant differences in weight loss, stool score, macroscopic and microscopic scores, weight change and percent weight loss compared to the control group. Additionally, there were no significant differences in Caspase-3, MPO, MDA and IL-10 levels. However, TNF-α levels were significantly higher in the propolis group compared to the control group, although no clinical or microscopic signs of colitis were observed. Nonetheless, TNF-α levels were lower in the propolis group compared to all colitis groups. Moreover, the weight change in the propolis group was similar to that of the control group.

The primary objective of our study was to investigate the differences between the acetic acid + propolis group and the acetic acid group. The stool score of the acetic acid group was significantly higher than that of the acetic acid + propolis group. Although not statistically significant, the other clinical parameters, such as weight loss score, macroscopic and microscopic scores, weight change, and percent weight loss, had lower median, minimum, and maximum values in the acetic acid + propolis group, providing evidence for the protective effect of propolis against colitis. The lower levels of pro-inflammatory cytokines TNF-α and tissue destruction products MPO and MDA in the acetic acid+propolis group compared to the acetic acid group indicate the cytoprotective activity of propolis in colitis at the biochemical level.

The limitations of our study include the lack of evaluation of the antibacterial activity of propolis and the difficulty in determining the optimal dose of rectal propolis. Moreover, the absence of a group administered only olive oil limits the evaluation of the effectiveness of olive oil. Additionally, we could not compare the effectiveness of oral and rectal propolis as there was no group given oral propolis.

5. Conclusion

Propolis is a well-known substance with anti-inflammatory properties and its low cost and ease of production make it an advantageous option compared to other drugs. Our study shows that propolis, which has previously been shown to have positive effects on colitis when taken orally, can also be used rectally for the treatment of colitis. The rectal route is preferred in colitis treatment due to the reduced risk of systemic side effects. While propolis and olive oil have similar anti-inflammatory activity on a biochemical level, our study suggests that propolis is superior to olive oil in terms of its clinical effectiveness. The literature contains numerous publications demonstrating the anti-inflammatory and antibacterial effects of propolis, as well as its protective effect against colitis, and our study supports these findings.

Statement of ethics

The study protocol was approved by the Cukurova University TTU-2022-14631

Conflict of interest statement

The authors declare that they have no financial conflict of interest with regard to the content of this report.

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Availability of data and materials

The data supporting this study's findings are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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