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Tianeptine exerts ameliorative effects on acetic acid-induced colitis as detected by anti-inflammatory and antioxidant markers in rats; the role of anti-LOX and radical scavenging activity

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ABSTRACT: Some patients with ulcerative colitis (UC) could benefit from antidepressant use, particularly when having concomitant depression or during the remission period. For this purpose, we investigated the therapeutic effects of Tianeptine (TT) on UC induced by acetic acid (AA) in rats from the perspective of both in vitro and in vivo anti-inflammatory and antioxidant activity. Within the *in vivo* experiments, the rats were divided into control (C), AA+ TT, and AA+ sulfasalazine (AA+SS) groups. Colitis was induced with the application of AA 5% intrarectally. Compared to indomethacin (21.42 µg/ml), SS showed an equally strong anti-inflammatory activity (21.97 µg/ml), while Tianeptine had a good anti-LOX activity (88.57 µg/ml) *in vitro*. Compared to standard Trolox (13 µg/ml) against ABTS radical, Tianeptine (238.60 µg/ml) and sulfasalazine (1727 µg/ml) showed free radical scavenging activity *in vitro*. AA caused a depletion in colonic GSH ($p<0.001$) and an increase in MDA, MPO, luminol, and lucigenin chemiluminescence levels ($p<0.01$- $p<0.001$) compared with the C group. Macroscopic and microscopic scoring were elevated in the AA group ($p<0.001$). The AA+ TT and AA+ SS groups showed improvement in GSH, MDA, MPO, and CL levels compared to the AA group ($p<0.01$- $p< 0.001$). Macroscopic and microscopic scoring decreased in the AA+ TT and AA+ SS groups ($p<0.001$) compared to the AA group. In conclusion, our study demonstrated that TT has a curative effect on UC based on its in vitro and in vivo anti-inflammatory and antioxidant activities. TT can be used in patients with concomitant colon inflammation and depression.

KEYWORDS: Ulcerative colitis; Depression; Tianeptine; Anti-5-lipoxygenase; Anti-ABTS; MDA; MPO; GSH

1. INTRODUCTION

Ulcerative colitis (UC), a form of inflammatory bowel disease (IBD), affects a large population globally [1]. Although the exact etiology of UC is unknown, it is thought that a mix of inherited and environmental factors contribute to the disease's onset [2, 3]. Mental health issues are a significant yet underappreciated or sometimes disregarded concern for patients with IBD [4]. Over the last two decades, it has been more usual to include antidepressant medication in the routine treatment of individuals suffering from functional gastrointestinal (GI) disorders, including IBD [5, 6]. Literature findings declare that adding antidepressant therapy to the routine treatment of individuals suffering from IBD diseases makes a positive contribution to the treatment of the disease [5, 6]. In addition to improving a person's mental health, antidepressant medications have also been shown to have anti-inflammatory effects. [7]. Anxiety and depression (together with psychotic and somatic symptoms) are common symptoms of GI patients, particularly UC. The bidirectional relationship between mental disorders and IBD activity (Brain-gut axis)

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has been suggested to have a potential therapeutic effect on gut health due to the use of antidepressants in the cure of anxiety and depression. [8]. According to research, many ailments, such as ulcers and inflammation, coexist with cognitive disorders like depression, or such diseases are caused by depression, or individuals suffering from these diseases become prone to depression over time [9]. Tianeptine (TT) which is approved for the treatment of major depressive disorder, is suggested as an effective therapeutic agent in the treatment of irritable bowel syndrome (IBS) [9]. In addition to its potential benefits for anxiety and IBS, TT is reported to be effective on somatic symptoms of depression, especially those related to digestion [10].

Leukotrienes (LT) are potent lipid mediators of inflammatory responses linked to the pathogenesis of acute and chronic inflammatory disorders, including IBD [11]. All LTs are produced from arachidonic acid by expressing arachidonate 5-lipoxygenase (5-LOX). It also promotes neutrophil infiltration in experimental colitis by expressing adhesion molecules [12]. There is no evidence in the literature that TT has anti-5-LOX activity. However, if it does, it could be a valuable therapeutic target for cases of UC and other inflammatory diseases accompanied by depression.

Although numerous studies have been conducted on the effects of TT on the brain [13], a limited number of experimental studies have reported that TT has antiulcer effects in the rat stomach [14, 15]. On the other hand, there is no scientific evidence of anti-inflammatory and antioxidant effectiveness in UC.

Some patients with ulcerative colitis (UC) could benefit from antidepressant use, particularly when having concomitant depression or during the remission period. On this basis, we investigated the therapeutic effects of TT on acetic acid (AA)-induced UC in rats in terms of both in vitro anti-5-lipoxygenase and antioxidant activity and in vivo oxidative stress, antioxidant and inflammatory markers, and tissue morphology.

2. RESULTS

2.1 Anti-Lipoxygenase Activity

Compared to the IC_{50} value of 21.42 µg/mL of standard indomethacin, SS with an IC_{50} value of 21.97 µg/mL showed equally strong anti-inflammatory activity. In comparison, TT with an IC_{50} value of 88.57 µg/mL exhibited a good anti-lipoxygenase (5-LOX) activity.

2.2. 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Radical Scavenging Activity

Compared to standard Trolox (13 µg/ml) against ABTS radical, TT (238.60 µg/ml) showed moderate antioxidant (radical scavenging) activity, while sulfasalazine (SS) (1727 µg/ml) showed weak antioxidant activity.

2.3. Oxidative Markers, Antioxidant Status and Inflammation

We demonstrated that AA caused a significant depletion in colonic glutathione (GSH) levels compared with the C group ($p < 0.001$), also a substantial increase in GSH levels was observed in AA+TT ($p < 0.001$) and AA+ SS ($p < 0.001$) groups (Figure.1a). The effect of TT was almost similar to the SS group (Figure 1a).

AA caused a significant elevation in colonic malondialdehyde (MDA) levels compared with the C group ($p < 0.01$). A significant decrease in MDA was observed in the AA+TT, and AA+SS groups compared to the AA group receiving physiological saline ($p < 0.01$). Additionally, no significant difference was observed in the AA+TT and AA+SS groups compared to the C group regarding MDA (Figure. 1b).

As an indicator of neutrophil immigration to the inflamed tissue, myeloperoxidase (MPO) activity was significantly higher in the colon tissues of the AA group than in the C group ($p < 0.001$). In the AA+TT and AA+SS groups, colonic MPO activities were significantly lessened to a level that was not different from the C group ($p < 0.001$) (Figure 1c).

Luminol and Lucigenin chemiluminescence (CL) levels in colon tissue exerted significant increases in the AA group compared to the C group ($p < 0.001$). This elevation in either luminol or lucigenin CL was diminished both in the AA+TT and AA+SS groups ($p < 0.001$), and these reduced CL levels were not different from that of the C group (Figure 1d and 1e).

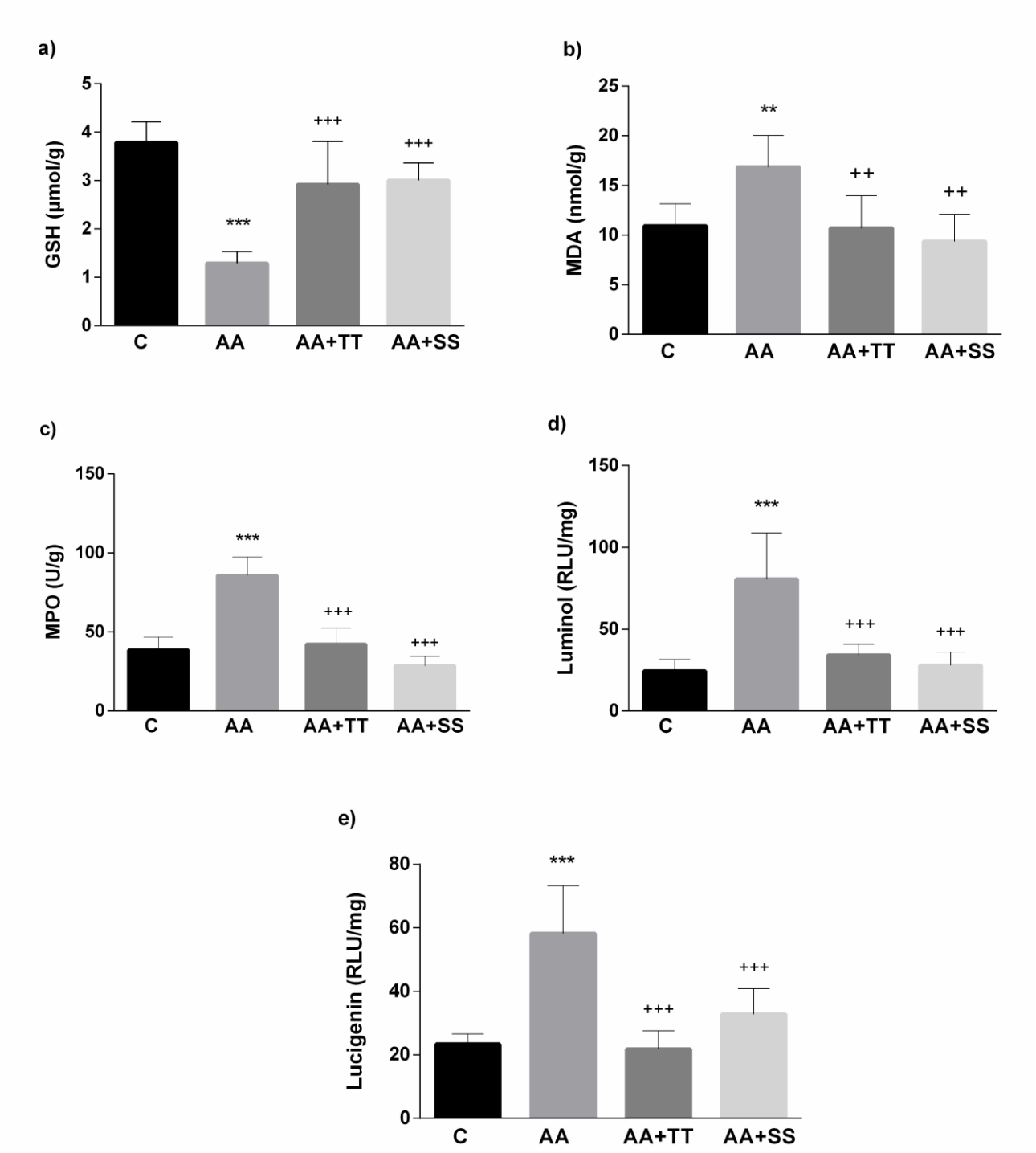

Figure 1. a) Glutathione (GSH) level, b) Malondialdehyde (MDA) level, and c) Myeloperoxidase (MPO) activity, d) luminol, and e) lucigenin chemiluminescence level in the colonic tissues of groups (n = 6 per group). Values are described as mean ± SEM.
C: sham + vehicle treatment, AA: acetic acid, TT: Tianeptine, SS: Sulfasalazine. **p < 0.01, *** p < 0.001; versus control group. ++p < 0.01, +++p < 0.001; versus acetic acid induction colitis group.

2.4. Macroscopic Appearance and Scoring

Macroscopic appearances of the removed colon tissues were scored (n=6) and photographed (Figure 2). The AA group had a higher overall macroscopic score (5.67 ± 0.5) compared to the C group (0.18 ± 0.1), AA+TT (1.83 ± 0.4), and AA+SS group (1.67 ± 0.3). The total macroscopic damage data obtained by evaluating the histopathologic changes in the colon tissues of the experimental groups are given in Figure 3a. The macroscopic damage score was found to be significantly increased in the AA group than in the C group ($p < 0.001$), and colonic damage was reduced macroscopically in both treatment groups (AA+TT and

AA+SS treated groups) (p <0.001). However, the macroscopic score level in both of the two treatment groups was still found to be significantly greater than the C group (p < 0.05) (Figure 3a).

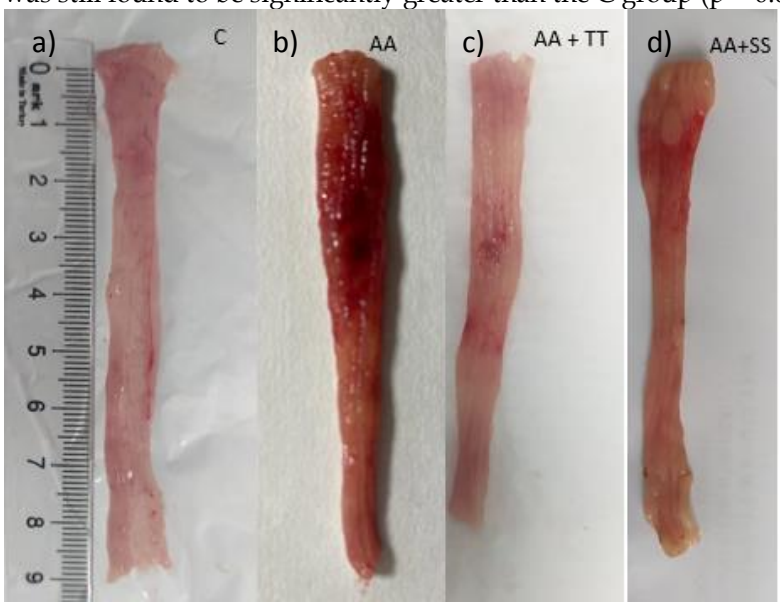

Figure 2. Macroscopic appearance of colonic tissues of a) control group (C), b) colitis group (AA), c) Tianeptine treatment group (AA+TT), d) Sulfasalazine treatment group (AA+SS) group, (n=6 per group).

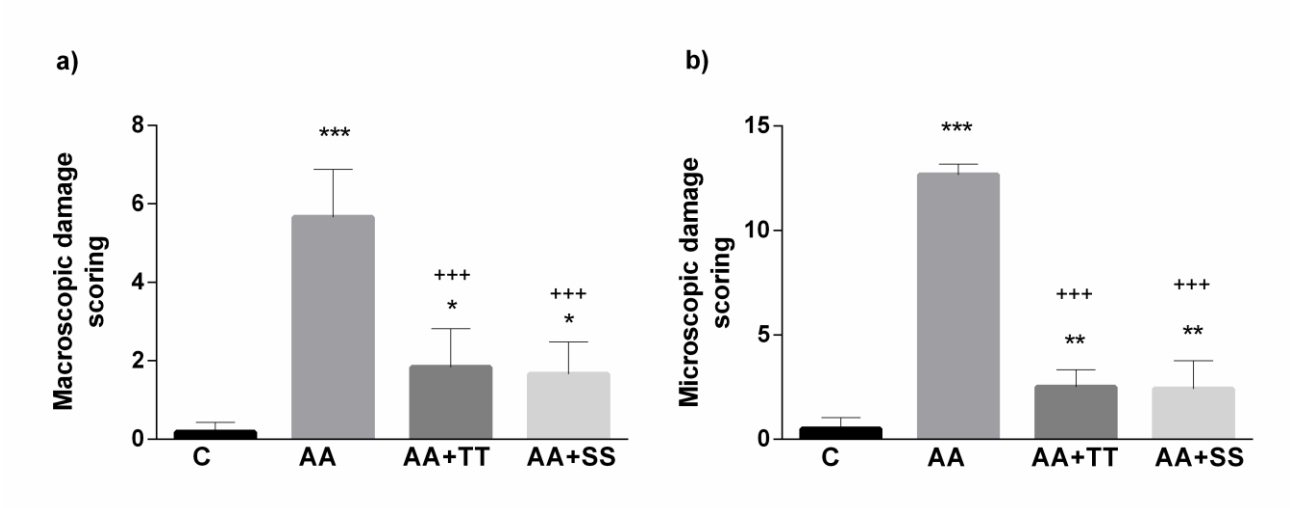

Figure 3. a) Macroscopic damage scoring, b) microscopic damage scoring of colon tissues of the groups (n = 6 per group), Values are described as mean ± SEM.

C: control, AA: acetic acid, TT: Tianeptine, SS: Sulfasalazine. *p < 0.05, **p < 0.01, *** p < 0.001; versus control group. + ++p < 0.001; versus acetic acid induction colitis group.

2.5. Microscopic Scoring, and Appearance

The AA group had a higher overall microscopic score (12.66 ± 0.2) compared to the C group (0.5 ± 0.2), AA+TT (2.5 ± 0.3), and AA+SS group (2.42 ± 0.5). The total microscopic damage data obtained by evaluating the histopathologic changes in the colon tissues of the experimental groups were given in Figure 3b. The high microscopic damage score noted in the colonic tissue of the AA group (p < 0.001) was significantly attenuated in the AA+TT and AA+SS groups (p < 0.001). However, the damage in groups AA+TT and AA+SS was still higher than in group C (p < 0.01, Figure 3b)(n=6).

The microscopic investigation of the sections of the C group stained with hematoxylin and eosin (H&E) showed normal colonic histology with regular epithelium, glands, and submucosa (Figure 4a). In the AA group, a high decrement of surface epithelial lining, loss of glands, extensive submucosal edema, and severe inflammatory cell migration were noticed (Figure 4b). In the AA+TT group, mucosal, glandular degeneration, submucosal edema, and mild inflammatory cell infiltration were lower than in the AA group (Figure 4c). Similar histological appearances, such as more regular epithelial surfaces and glands, were observed in the AA+SS group (Figure 4d) (n=6).

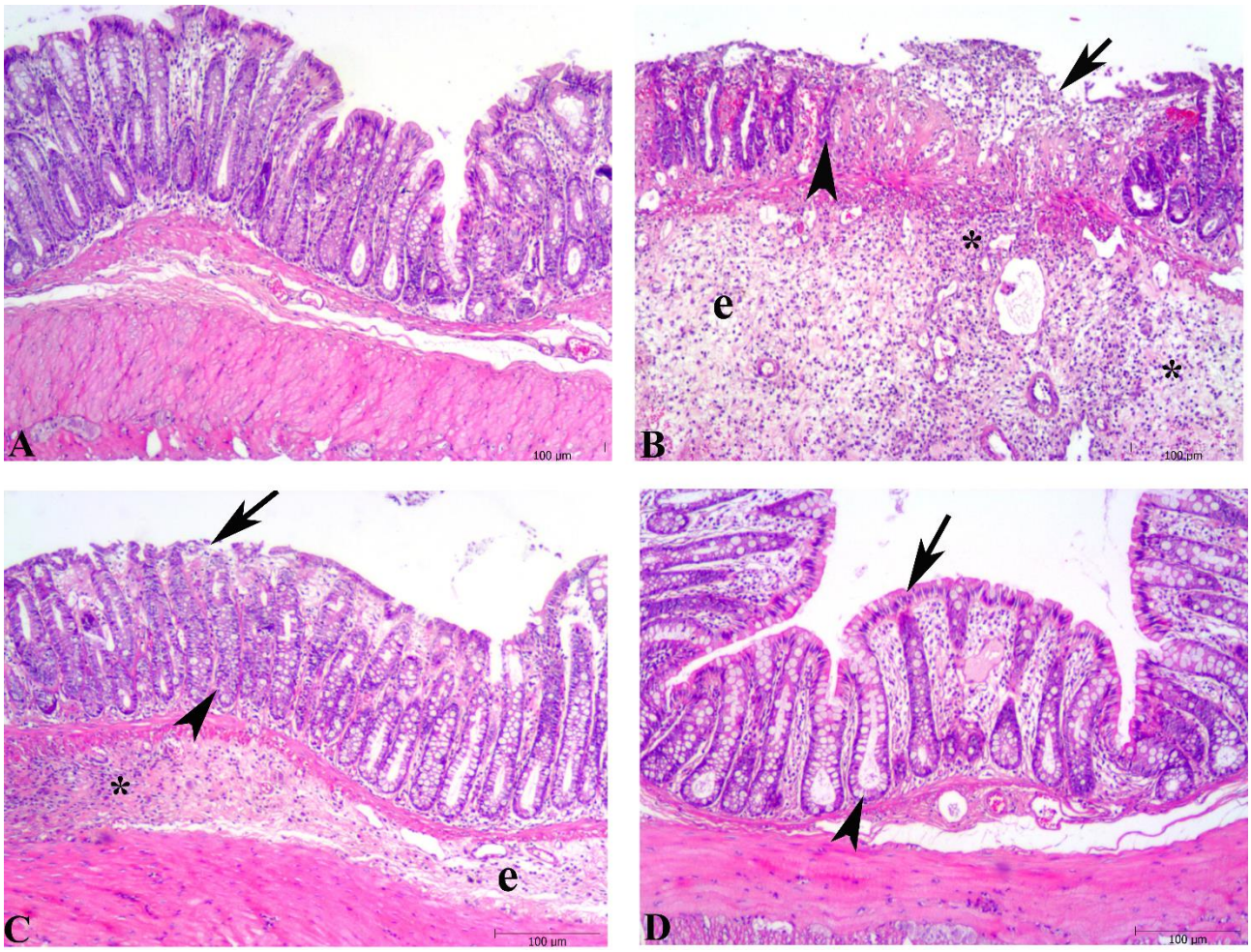

Figure 4. Representative light micrographs of the colonic tissue sections stained with Haematoxylin and Eosin (H&E). a. Regular surface epithelium, glands, and submucosal area in control tissues. b. Massive loss of surface epithelium (arrow) and glands (arrowhead), submucosal edema (e), Severe inflammatory cell infiltration (*) in acetic acid-induced colitis groups. c. Decreased mucosal (arrow) and glandular (arrowhead) degeneration, degreased submucosal edema (e), and moderate inflammatory cell infiltration (*) in the TT-treated colitis groups. d. Regular surface epithelium (arrow) and glands (arrowhead) in the SS-treated colitis groups: scale bar, 100 µm. (n=6 per group).

3. DISCUSSION

The results of our study reveal that TT heals colonic damage. TT has good anti-LOX and moderate antioxidant activity in vitro. TT decreased MPO activity MDA level and increased GSH level in rats in vivo. Decreased CL levels reflect the suppressive effect of Tianeptine and SS on ROS formation. Moreover, significant improvements in macroscopic and microscopic damage, as well as colitis findings, were observed with TT and SS treatments.

5-LOX is an important enzyme in the production of pro-inflammatory leukotrienes (LT), which engage in a prominent role in numerous inflammatory conditions [16]. Therefore, 5-LOX inhibitors are often applied to study as therapeutic targets in inflammatory disease models including ulcerative colitis [17, 18]. Treatment with a 5-lipoxygenase inhibitor, PF-5901, resulted in a substantial lessening of the strictness of colitis [19]. The degree and severity of histological signs of colonic damage were reduced in 5-LOX-deficient mice that developed colitis with dinitrobenzene sulphonic acid (DNBS), compared with 5-LOX wild-type mice (which have the LOX enzyme) [12]. In our study, tianeptine was shown to have good anti-LOX activity in vitro for the first time. On the other hand, a limitation of our study is that we could not examine the anti-cyclooxygenase enzyme activity which is another important enzyme in the production of cyclooxygenases of TT.

In parallel, our results of decreasing colonic damage score also support the above-mentioned studies. ABTS is the most popular and commonly used among numerous methods for antioxidant activity

estimation due to their ease, speed, sensitivity, and stable radicals [20]. ABTS, in particular, has been reported as a decolorization assay applicable to both lipophilic and hydrophilic antioxidants [21]. In the current study, TT showed good antioxidant activity against ABTS radicals. Therefore, it may be considered that the antioxidant effect contributes to the effect of Tianeptine against colitis in addition to its anti-inflammatory effect. At the same time, TT, unlike SS, contains a chloro group in its structure. The presence of chloro groups in a molecule has been reported to increase the antioxidant effect against ABTS radical [22]. Therefore, the chloro group may have made an essential contribution to the antioxidant effect of TT. On the other hand, in the present study, although SS showed weak antioxidant activity *in vitro* measurements when it enters the organism, it scavenges free radicals and reduces oxidative stress. The main reason for this is that SS decomposes into 5-aminosalicylic acid (5-ASA), a salicylic acid derivative in the intestine. 5-ASA is known to have strong antioxidant effects [23]. In the present study, luminol and lucigenin-enhanced CL, and LP measurements, which were found to be decreased in both TT and SS treatment groups, also support the antioxidant activities of these substances.

The AA-induced UC model closely mimics human IBD in terms of pathological and inflammatory mediators. [24]. AA induction initiates damage to colon tissue, causing inflammation. In addition, neutrophils and other immune system cells migrating into the injured colon mucosa produce large amounts of ROS, causing oxidative stress, and increasing inflammation severity [25, 26]. In this current study, AA induction significantly increased MPO activities in colon tissue compared to the control ones. Neutrophil stimulation causes excessive scavenging of free radicals and thus releases products that react with MPO and cause tissue damage [25, 26]. Furthermore, an increase occurs in lipid peroxidation and a consequent decrease in GSH levels. Following previous studies, our study has also revealed that GSH levels in the colon diminish in AA-induced colitis [27]. Tianeptine has been reported to exert anti-inflammatory effects. This effect was realized by decreasing the MDA level and increasing the activity of antioxidant enzymes in the brains of rats exposed to a chronic, unpredictable stress model [28]. In another study, MDA and GSH levels in a rat model of seizure-induced oxidative stress and renal ischemia-reperfusion injury were reported to be improved by TT [13, 29]. TT also significantly prevented decreased GSH content and increased MDA and MPO levels in indomethacin-induced gastric ulcers in rats [29]. In the findings of this study, the increase in GSH content and the decrease in MDA and MPO activation were associated with TT's antioxidant and anti-inflammatory effects. Cuzzocrea et al. (2005) also observed significant decreases in the degree of colon damage and MPO activity in 5-LOX-deficient mice [12]. Our results are consonant with the consequences of this study. Accordingly, it can be suggested that tianeptine reduces MPO levels by reducing 5-LOX activity.
The luminol & and lucigenin-enhanced CL assessment protocol enables us to quantify the ROS level in our study (Luminol detects hydrogen peroxide, hydroxyl, hypochlorite, peroxynitrite, and lipid peroxyl radicals, while lucigenin detects superoxide radicals). The present CL data indicated that AA causes toxic substances sensed by both probes, which can excessively damage the cells in the colon mucosa. The raises in all ROS in the colon were significantly decreased in AA+ tianeptine and AA+ SS groups. It has been previously reported that TT reduces ROS levels in ketamine-induced heart injury rat models [30]. Our results reflect the decrease in the amount of ROS. We suggest that this is due to TT's ABTS radical scavenging activity.
Studies have shown that TT (opiate) binds specifically to the μ-receptor subtype and stimulates μ-receptors even when taken orally [31]. Opiate agonists are primarily known as analgesics, but μ-agonists in particular also have anti-inflammatory activity [32, 33]. The literature indicated that morphine, a μ- receptor agonist, a given in vivo, suppresses the major cell types in the adaptive and innate immune system, including macrophages and polymorphonuclear leukocytes, and reduces phagocytic activity and production of ROS and prostaglandins [34]. In the current study, the decrease in MPO level and ROS supports previous studies in this respect because TT is also an μ-receptor agonist.

Chojnacki et al. (2011) reported that TT positively affects the psychological and somatic conditions of patients with UC and recommended using Tianeptine as an adjuvant drug during the remission period in UC [4]. As demonstrated in this study, considering TT's good anti-inflammatory and antioxidant properties and its morphological and biochemical healing effects on colitis, we can suggest that it can be used primarily in UC remission or patients with depression and UC.

4. CONCLUSION

In conclusion, our study demonstrated the curative effect of TT on UC based on its anti-inflammatory and antioxidant activities in vitro and in vivo. TT can be used in UC patients with concomitant depression or during the remission period.

5. MATERIALS AND METHODS

In the current study, *in vitro,* ani-LOX activity and ABTS free radical scavenging activity measurements were first executed, and then *in vivo,* experiments were performed (Figure 5).

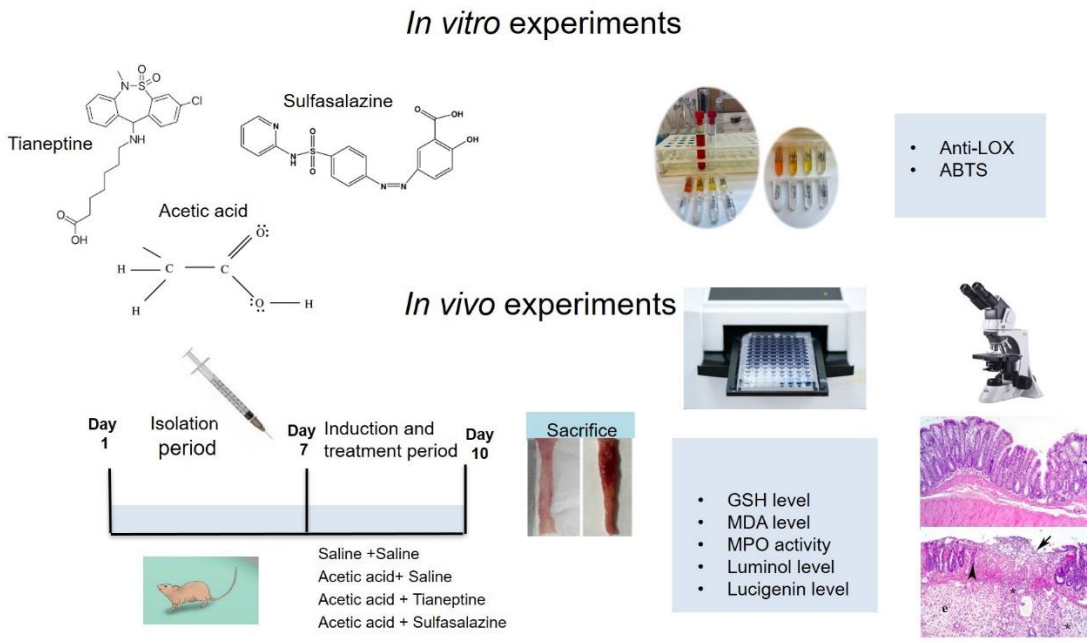

Figure 5. Experiment flow diagram.

In vitro, anti-LOX activity and ABTS free radical scavenging activity measurements were first executed, and then *in vivo*, experiments were performed. After the habituation period, the rats were divided into four groups (n=6):

Physiological saline (1ml/ i.r) + saline (1ml/ i.p) treatment group (C group); AA (1ml/ i.r) + physiological saline (1ml/ i.p) treatment group (AA group); group: AA (1ml/ i.r) + 50 mg/kg bwt Tianeptine (1ml/ i.p) treatment group (AA+TT group); AA (1ml/ i.r) + 100 mg/kg bwt Sulfasalazine (1ml/ i.p) group (AA+SS group). All rats were euthanized under anesthesia. The following procedures were applied to the dissected colon tissues: MPO activity, GSH, MDA, luminol and lucigenin level, macroscopic and microscopic damage scoring, and histologic evaluation (n=6).

MPO: Myeloperoxidase, GSH: Glutathione, MDA: Malondialdehyde, Anti-LOX: Anti-Lypoxigenase, ABTS: 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid.

5.1. Measurements of Anti-LOX Activity

The anti-inflammatory activity of TT and SS samples was determined using the 5-LOX enzyme by Yildirim et al. (2019) [35]. Ten µl of sample stock concentrations (5000-9.77 µg/ml) or indomethacin as a standard (250-0.49 µg/ml) was added to alcohol (20 µl), clean water (20 µl), and sodium borate buffer liquid (25 µl, 0.1 M, pH 9) followed by the addition of type V soybean LOX solution (25 µl) in a buffer (pH 9, 20,000 U/ml). After incubating at 25 °C for 5 min, linoleic acid liquid (0.6 mM, 100 µl) was added and carefully melded. The absorbance values at 234 nm were recorded for 6 min. The assay was done three times, with the findings given as IC50

5.2. Determination of ABTS Radical Cation Scavenging Activity

For the production of ABTS radical cation used in the determination of the total antioxidant capacity of the TT and SS samples, 7 mM ABTS was mixed with 2.45 mM potassium persulfate and the reaction was allowed to complete at room temperature (23 °C) for 16 h in the dark. The ABTS+ solution was diluted with analytically pure 96% ethanol solvent to an absorbance of 0.700 ± 0.050 at 734 nm. Ten µl of the solution of each concentration (5000-9.77 µg/ml) was transferred to the microplate wells 190 µl of ABTS+ solution was added. The solution was kept at room temperature for 30 min and then measured at 734 nm. Trolox was standard, and the results were expressed as IC50 values [35].

5.3. Animals and Experimental Design

Sprague Dawley female rats (200–250 g) were provided by the Marmara University Application and Research Centre for Experimental Animals (200-250 g). The rats were housed in an aired room with a light-dark cycle (12:12), ensuring a temperature of 22 ± 2 °C and a specific humidity of 65-70%. The study followed guidelines authorized by the Marmara University Animal Experiments Local Ethics Committee (Protocol number: 14.2023mar; Date: 2023). All experimental practices concerning rats were performed following "The Guide for the Care and Use of Laboratory Animals" (www.nap.edu/ catalog/5140.html). After the habituation period (for 7 days), rats were divided into four groups of six rats each at random: 1. C group: physiological saline (1mL/ i.r) + physiological saline (1ml/ i.p) treatment group (as sham + vehicle treatment group), 2. AA group: AA (1mL/ i.r) + physiological saline (1ml/ i.p) treatment group, 3. AA+ TT group: AA (1ml/ i.r) + TT (50 mg/kg bwt, 1mL/ i.p) (Stablon, Servier Turkiye) treatment group, 4. AA+ SS group: AA (1ml/ i.r) + SS (100 mg/kg bwt, 1mL/ i.p) (Salazopryn, Pfizer Turkiye) group. The selected tianeptine dose was taken from Han et al.'s study, which observed that tianeptine had positive effects on neuropathic pain [36]. The selected SS dose was taken from Sakthivel, et al.'s study displaying that SS has anti-inflammatory and antioxidant effects [37]. All sets of treatment regimens were given intraperitoneally and given out as a single dose daily. All injections were started immediately after colitis and treatments were given for a total of 3 days, at the same time each day. All rats were euthanized using a high dose of anesthesia (Sodium pentothal, 50 mg/kg bwt, i.p, I. Ulagay Turkiye) 72 hours after colitis induction.

5.4. Induction of Ulcerative Colitis

After 8 hours of fasting, the rats under ether anesthesia were given 1 mL of 5% (v/v) AA in 0.9% physiological saline (pH:3) intrarectally using an 8-cm long soft silicone cannula for 30 s (applied at a 30% incline). After this application, the rats were taken back to their cages. The C group was administered the same volume of physiological saline using the same method [38].

5.5. Macroscopic assessment of UC severity

The distal 8 cm of the colon was cut and cleaned, rinsed with warm physiological saline, and wiped. Macroscopic scoring was achieved using the criteria described in Table 1 to assess colonic mucosal damage [39].

Table 1. Macroscopic score criteria

Score	Appearance
0	Normal appearance
1	Focal hyperemia, no ulcers
2	Ulceration without hyperemia and bowel wall thickening
3	Ulceration with inflammation at one site
4	Two or more sites of ulceration and inflammation
5	Major sites of damage extending more than 1 cm long length of the colon
6-10 (Maximum score 10)	Score is increased by 1 for each additional cm of damage beyond 2 cm

5.6. Assessment of Oxidative markers, Antioxidant Status and Inflammation

The colon samples (0.2-0.5 g) were mixed carefully for 30 s with trichloroacetic acid (10%, 10 mL) and then centrifuged at 704 g for 15 min (at 4 °C). The supernatant was then removed and centrifuged again at 10000 rpm for 8 min at 4 °C. Glutathione (GSH) levels were determined by modifying the Ellman method and the results were expressed as µmol/g tissue [40]. Malondialdehyde (MDA) level was applied to the procedure previously performed by Buege et al. and the results were expressed as nmol/g tissue [41]. Myeloperoxidase (MPO) activity was measured by H_2O_2-dependent oxidation of o-Dianisidine dihydrochloride at 37 °C, and the results were expressed as units/g tissue [42]. Chemiluminescence (CL: luminol and lucigenin) levels (rlu/mg), markers of ROS formation, were measured using a luminometer (Junior LB 9509, EG&G Berthold, Germany) and the technique reported in previous studies [43].

5.7. Microscopic assessment of UC severity

For general morphological analysis, colon tissue sections (5 µm) were colored with H&E, evaluated with a microscope (Olympus CX21, Tokyo, Japan), and photographed (Olympus, Tokyo, Japan, BX51). Microscopic scoring was performed according to the criteria of a previous study [44].

5.8. Statistical analysis

After verifying the normal distribution of data by the Kolmogorov-Smirnov test, followed by analysis of variance (ANOVA), Tukey's multiple comparison test was applied for comparisons between groups. Prism 6.0 program (GraphPad Software, San Diego, CA, USA) was used for statistical analyses. Results were described as mean ± standard error of the mean (SEM), and $p < 0.05$ was considered significant.

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