*Inflammation, Vol. 42, No. 6, December 2019 (© 2019)*
DOI: 10.1007/s10753-019-01068-0

ORIGINAL ARTICLE

# Potential Anti-Inflammatory Effect of Escitalopram in Iodoacetamide-Induced Colitis in Depressed Ovariectomized Rats: Role of α7-nAChR

**Salah A. Abdo,[1,2] Walaa Wadie,[1] Rania M. Abdelsalam,[1] and Mahmoud M. Khattab[1]**

*Abstract*— Escitalopram, a drug of choice in the treatment of depression, was recently shown to possess an anti-inflammatory activity. The aim of the present study was to elucidate the effect of escitalopram on peripheral inflammatory cascades in iodoacetamide-induced colitis associated with depressive behavior in ovariectomized rats. Moreover, the role of α-7 nicotinic acetylcholine receptor in mediating the anti-colitic effect of escitalopram was examined using a nicotinic receptor antagonist methyllycaconitine citrate. Colitis was induced by intracolonic injection of 4% iodoacetamide in ovariectomized rats. Escitalopram (10 mg/kg/day, i.p.) was then injected for 1 week and several parameters including macroscopic (colon mass index and ulcerative area), microscopic (histopathology and scoring), and biochemical (myeloperoxidase and tumor necrosis factor-α) were determined. Colitis induction in ovariectomized rats resulted in a marked increase in colon mass index, ulcerative area, histopathological scoring, myeloperoxidase activity and tumor necrosis factor-α levels. These effects were ameliorated by escitalopram, even in the presence of methyllycaconitine indicating that α-7 nicotinic acetylcholine receptor does not mediate the anti-inflammatory effect of escitalopram. The present study revealed the beneficial effect of escitalopram in iodoacetamide induced colitis in ovariectomized rats and suggests that it may represent a new therapeutic agent for the treatment of inflammatory bowel disease, especially in patients with or at high risk of depressive behavior.

**KEY WORDS:** Escitalopram; colitis; depression; α7-nAChR; iodoacetamide; ovariectomy; methyllycaconitine.

## INTRODUCTION

Depression is more common in patients with inflammatory bowel disease (IBD) than other chronic diseases such as diabetes, cancer and cystic fibrosis [1], and the use of antidepressant may have a beneficial effect on IBD activity [2]. IBD patients who use antidepressants had a significantly lower relapse rate as compared to nonusers [3]. Selective serotonin reuptake inhibitors (SSRIs) are the most widely used group for

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s10753-019-01068-0) contains supplementary material, which is available to authorized users.

[1] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt
[2] To whom correspondence should be addressed at Department of Pharmacology and Toxicology, Faculty of Pharmacy, Cairo University, Cairo, Egypt. E-mail: salah292006@hotmail.com

treatment of depressive symptoms [4]. The psychological problems in IBD patients were effectively improved by SSRIs (citalopram, sertraline) [5]. Fluoxetine was found to protect against colitis in a randomized controlled trial [6]. Escitalopram (ESC) showed anti-inflammatory and antidepressant effects in a lipopolysaccharide-induced inflammatory model of depression [7]. However, the anti-inflammatory effect of ESC in colitis has not yet been investigated. In a murine model of colitis, the vagus nerve provided tonic inhibition of acute inflammation [8] and the secretion of proinflammatory cytokine can be suppressed through activation of α-7 nicotinic acetylcholine receptor (α7-nAChR) on macrophages [8–10]. Selective α7-nAChR agonist was found to protect against experimental colitis induced by trinitrobenzene sulfonic acid in mice [11]. The aim of the present study is to investigate the anti-inflammatory effect of ESC on colitis in depressed rats and to throw some light on the role of α7-nAChR, using α7-nAChR antagonist, namely, methyllycoconitine (MLA).

## MATERIALS AND METHODS

### Animals

Forty young adult (3 months old) female rats, weighing 150–200 g, were obtained from the animal house of Faculty of Pharmacy, Cairo University, and were left to acclimatize for 1 week before subjecting them to experimentation. They were provided with a standard pellet diet and given water *ad libitum*. The animals were kept at a temperature of 22 ± 3 °C and a 12-h light/dark cycle as well as a constant relative humidity throughout the experimental period. The investigation complies with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85–23, revised 2011) and was approved by the Ethical Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University (Permit Number: PT 1675).

### Drugs

ESC hydrochloride was obtained from Saba'a International Company for Pharmaceutical and Chemical industry (Industrial area, Cairo, Egypt). Iodoacetamide was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). MLA was from Tocris Bioscience, Bristol, UK.

### Experimental Design

Before the experiments, forced swimming test (FST) was used after 2 weeks of ovariectomy to confirm the depressive state. For this purpose, two groups of animals were involved (ovariectomized (OVX) and sham-operated (SO) rats) (data provided as supplementary). After the depressive state has been confirmed, the animals were randomly allocated into 5 groups, 8 rats each, as follows: group 1, normal control group was subjected to SO and single intrarectal instillation of 1% methylcellulose and then received normal saline (i.p.) for 7 days. Group 2, OVX + colitis control group was subjected to ovariectomy, and single intrarectal instillation of 4% iodoacetamide [12] and received normal saline (i.p.) for 7 days. Group 3, OVX + colitis+ESC group was treated as group 2 but received i.p. injection of ESC (10 mg/kg/day) [13] for 7 days. Group 4, OVX + colitis+MLA group was treated as group 2 but received i.p. injection of MLA in a dose of 5.6 mg/kg/day [14] for 7 days. Group 5, OVX + colitis+ESC + MLA group was treated as group 2 but received both MLA and ESC for 7 days. The MLA was given 30 min before ESC [14].

At the end of the experiment, the rats were sacrificed by cervical dislocation under light anesthesia and the distal 8 cm of the colon was excised, opened longitudinally, rinsed in ice-cold normal saline, cleaned of fat and mesentery, blotted on filter paper, and weighed for subsequent macroscopic, microscopic, and biochemical assessment of colitis.

### Surgical Procedure of Ovariectomy

Animals were bilaterally ovariectomized under intraperitoneal ketamine (50 mg/kg) and xylazine (10 mg/kg) anesthesia. A small incision through the skin, connective tissue and the muscle layer, was made in the region from the hip to the lowest rib on both sides. The ovaries were exteriorized with the associated fat pad and fallopian tube. A suture knot was made around blood supply to the ovaries in which a hemostatic clamp was applied. After that, the ovaries were cut away and discarded. The muscle and skin layer were sutured, and the wound was topically treated with betadine and antibiotic spray. After surgery, animals were wrapped in a piece of cotton and kept in a heated place for at least 2 h. One week was given for the animals to recover from the operation, follow up, and to detect any complication of the surgery [13].

### Induction of Experimental Colitis

Before induction of colitis rats were fasted for 24 h but had free access to drinking tap water. The rats were sedated by diethyl ether and a catheter placed 8 cm proximal to the anus was inserted to the colon to infuse 0.1 ml of 4% iodoacetamide dissolved in 1% methylcellulose into the colon [12].

### Assessment of Depression

*Forced Swimming Test.* The rats were placed individually in a plastic cylinder (50 cm height × 20 cm diameter) containing tap water maintained at room temperature during testing (25 ± 2 °C), 30 cm deep, so that rats could not support themselves by touching the bottom of cylinder with their paws or tails. The test was carried out on 2 days. The first day is training day, in which rats were trained to swim for 10–15 min. On the second day, rats were re-exposed to the forced swimming under the same conditions from the previous day for 5 min. The 5-min test was videotaped and the time during which the animal floats on the water surface making only the movements which were necessary to keep its head above the water was recorded as total immobility time [15].

*Open Field Test.* Locomotor activity was assessed using the open field test 2 weeks after the surgery (on day 15). Each rat was placed gently in the central area of a square wooden box (80 × 80 × 40 cm) with red walls and black smooth polished floor divided by white lines into 16 equal squares (4 × 4). The rats were allowed to freely explore the area for 5 min. During this period, the number of squares crossed was recorded [16]. To eliminate possible bias due to odors left by previous rats, the floor and walls were cleaned after each tested animal.

### Assessment of Colitis

*Macroscopical Parameters.* Mucosal damage was assessed by measuring the ulcerative area ($cm^2$). The ratio of colon weight (mg) to body weight (g) was taken as the colon mass index that was used as a measure of colonic edema degree and severity of inflammation [17].

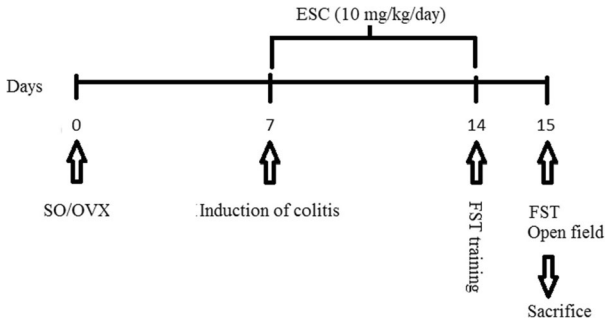
*Histopathological Assessment.* The colon segment was fixed in 10% formalin and preserved for histological examination. Transverse sections (4–6 μm) were prepared from paraffin-embedded colon segments from each animal. Each section was stained with hematoxylin and eosin (H&E) and examined under a light microscope and graded individually by a pathologist blinded to the treatment regimen. Each section was assigned a damage score between 0 and 3 for each of five parameters, namely, mucosal necrosis, mucosal inflammatory cells infiltration, submucosal inflammatory cells infiltration, fibrosis, and submucosal oedema. The scores for the five parameters measured for each rat were summed to obtain the "total histology score," being maximally 15 (3 as the maximum for the 5 parameters examined). The data were then represented using a box plot [12].

*Biochemical Measurements.* The colon segment was homogenized in ice-cold normal saline to obtain a 10% homogenate. An aliquot of colon homogenate was mixed with an equal volume of 100 mmol/L phosphate buffer pH 6 containing 1% hexadecyltrimethylammonium bromide. The mixture was freeze-thawed, sonicated for 10 s, and centrifuged at 10000 rpm for 15 min at 4 °C. The supernatant was used for spectrophotometric estimation of myeloperoxidase (MPO) activity [18]. Another aliquot of colon homogenate was used for assaying tumor necrosis factor-α (TNF-α), using specific commercial enzyme-linked immunosorbent assay (ELISA) kits obtained from R&D Systems (GmbH, Wiesbaden, Germany) (Scheme 1).

*Statistical Analysis.* All data obtained, except for histological scores, were expressed as means ± SEM and analyzed using one-way-analysis of variance test (one-way ANOVA) followed by Tukey's Kramer multiple comparison tests. Histological scores were presented as median and analyzed using Kruskal-Wallis test followed by Dunn's test. Statistical analysis was performed using GraphPad Prism software, version 6.01 (GraphPad Software Inc., San Diego, CA). For all the statistical tests, the level of significance was set at $p < 0.05$.

## RESULTS

*Forced Swimming Test.* OVX and intrarectal administration of iodoacetamide resulted in depressive-like behavior appeared as increased immobility time by 159%, when compared to SO group ($p$ value < 0.0001) this was normalized by the administration of ESC. The significant




**Scheme 1.** Schematic representation of the experimental design. SO: sham operation, OVX: ovariectomy, ESC: escitalopram, FST: forced swimming test.

effect of ESC was also noticed in groups received MLA (Fig. 1a).

*Open Field Test.* Two weeks after OVX, locomotor activity was assessed to determine whether the changes induced by ESC in the FST could be due to non-specific effects on locomotor activity. No significant difference in the number of squares crossed during a 5-min test was elucidated among the groups (Fig. 1b).

*Macroscopical Parameters of Colitis.* OVX and intrarectal administration of iodoacetamide resulted in a significant decrease in body weight of animals, as compared to the normal control group. Moreover, OVX and colitis induction led to a significant increase in colon mass index and ulcerative area as compared to normal group an effect that was ameliorated by ESC. Furthermore, ESC effect on the aforementioned parameters was also observed in groups receiving MLA (Fig. 2).

*Histopathological Assessment.* OVX + colitis group showed detachment of surface epithelial cells, focal aggregation of inflammatory cells, thickening of muscularis mucosa, and hypertrophy of surface epithelial cells with diffuse infiltration of inflammatory cells. This was reflected in a marked increase in total histology score. Rats treated with ESC alone or with MLA showed marked decrease in cellular infiltration and restoration of the normal structure of tissue with significant reduction in total histology score, as compared to OVX + colitis group ($p$ value < 0.0001) (Fig. 3).

*Myeloperoxidase Activity.* OVX rats with iodoacetamide-induced colitis showed **a** significant increase in colonic MPO activity, as compared to normal group. Treatment with ESC significantly decreased this rise ($p$ value < 0.0001), an effect that also noticed in rats received both ESC and MLA ($p$ value < 0.0001) (Fig. 4a).

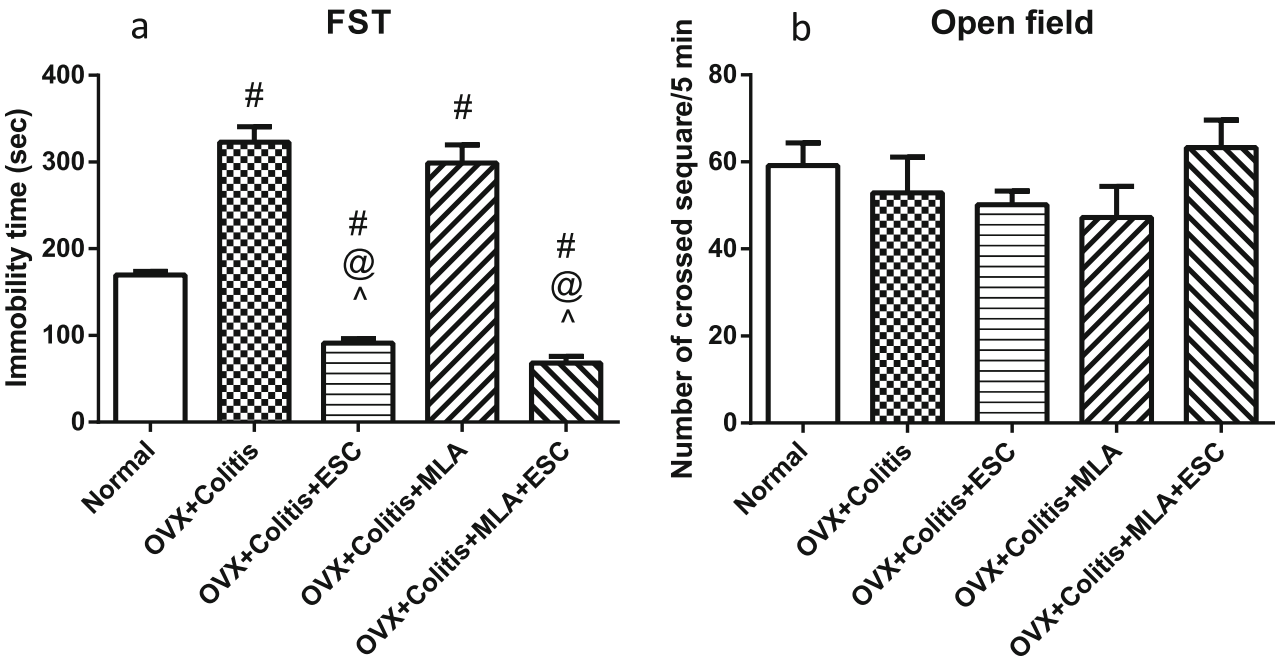



**Fig. 1.** Effect of escitalopram (ESC) and/or methyllycoconitine (MLA) on (**a**) forced swimming test (FST), and (**b**) open field test, in ovariectomized (OVX) rats with iodoacetamide-induced colitis. Data are expressed as means ±SEM of 8 animals. # vs normal group, @ vs OVX+colitis group, ^ vs OVX+colitis+MLA group (One-way ANOVA followed by Tukey-Kramer multiple comparisons test; $p<0.05$).

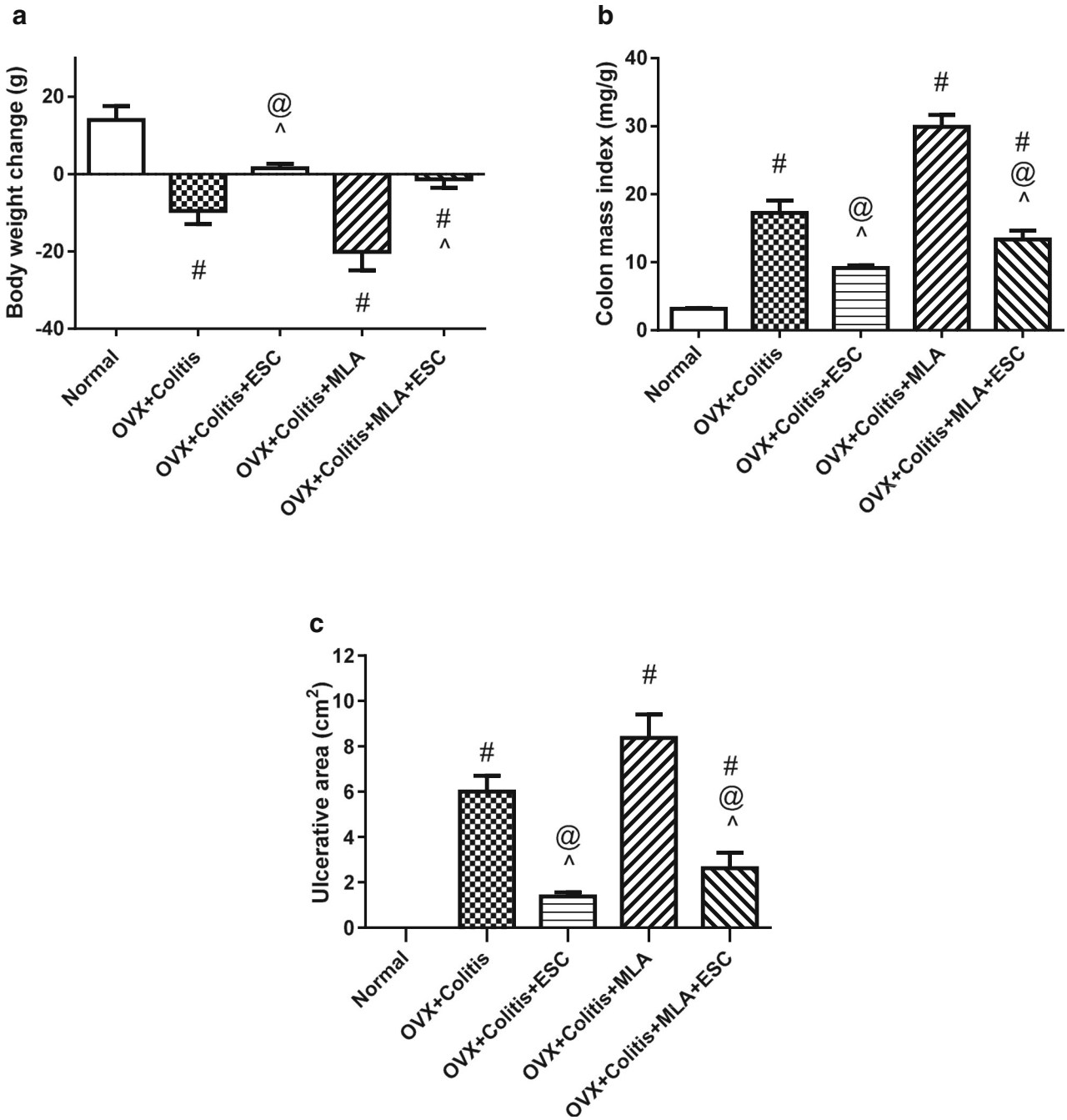



**Fig. 2.** Effect of escitalopram (ESC) and/or methyllycoconitine (MLA) on (**a**) body weight change, (**b**) colon mass index, and (**c**) ulcerative area in ovariectomized (OVX) rats with iodoacetamide-induced colitis. Data are expressed as means ± SEM of 8 animals. # *vs* normal group, @ *vs* OVX + colitis group, ^ *vs* OVX + colitis+MLA group (one-way ANOVA followed by Tukey-Kramer multiple comparisons test; $p < 0.05$).

*Tumor Necrosis Factor-α Concentration.* As shown in Fig. 4b, colonic TNF-α levels were significantly increased in OVX + colitis control group, as compared to normal group. Treatment with ESC resulted in a significant reduction in colonic levels of TNF-α, compared to OVX + colitis control group ($p$ value < 0.0001), even in the presence of MLA ($p$ value < 0.0001).

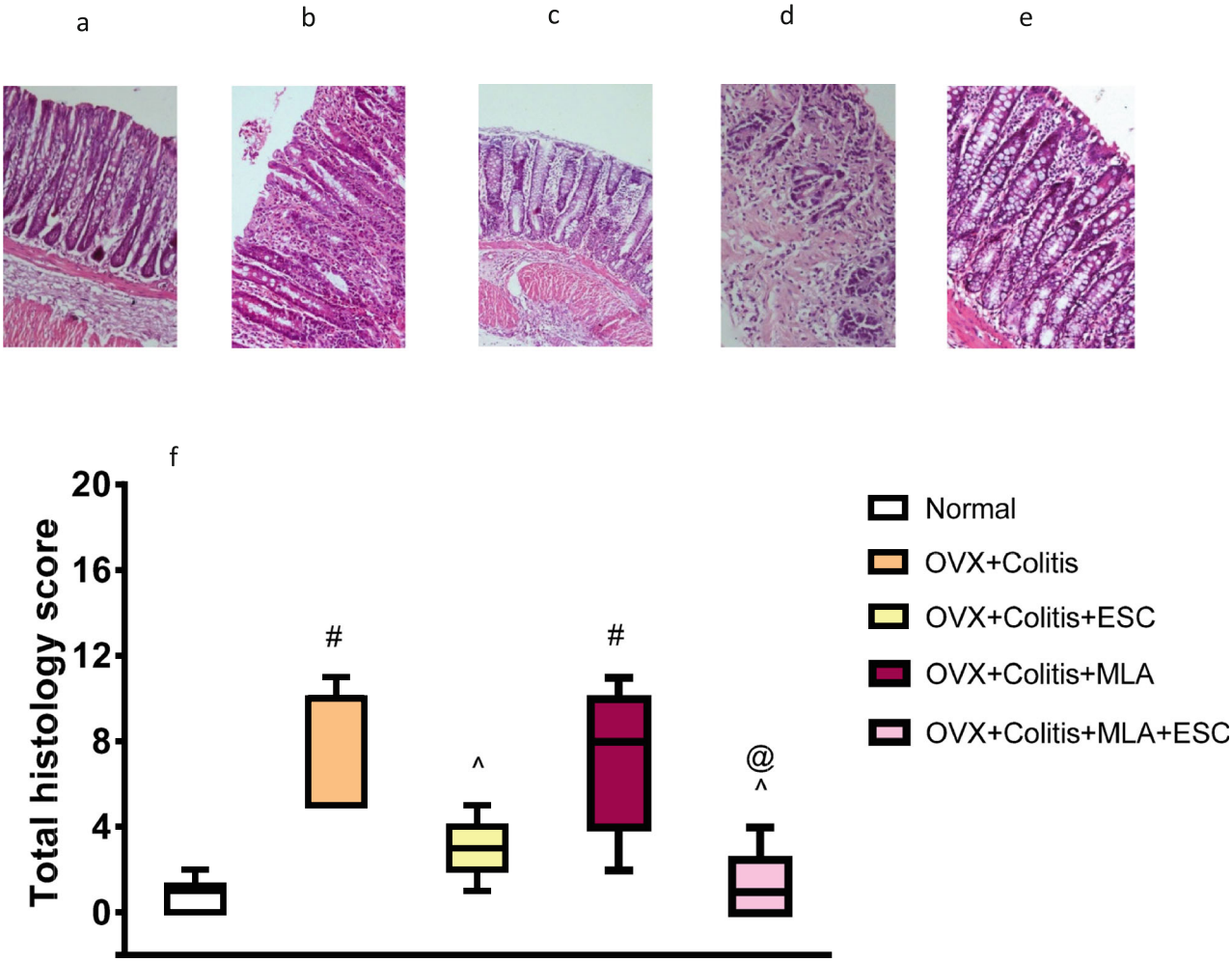



**Fig. 3.** Effect of escitalopram (ESC) and/or methyllycoconitine (MLA) on histopathological changes of rat colon in ovariectomized (OVX) rats with iodoacetamide-induced colitis. **a** Normal control rat: normal histological structure of mucosa. **b** OVX + colitis control rat showed detachment of surface epithelial cells, focal aggregation of inflammatory cells, thickening of muscularis mucosa, and hypertrophy of surface epithelial cells with diffuse infiltration of inflammatory cells. **c** OVX + colitis + ESC-treated rats showed a marked decrease in cellular infiltration and restoration of normal structure of tissue. **d** OVX + colitis + MLA-treated rats showed massive diffused infiltration of inflammatory cells with fibrous tissue in mucosa and submucosa. **e** OVX + colitis + ESC + MLA-treated rat showed marked decrease in cellular infiltration and noticeable regeneration of surface epithelial cells. (H&E, × 200). **f** Total histology score, data are expressed as box plots of the median of at least six animals. # $p \leq 0.05$ *vs.* normal control, @ *vs* OVX + colitis group, ^ *vs* OVX + colitis+MLA group.

## DISCUSSION

The potential anti-inflammatory effect of ESC on iodoacetamide-induced colitis associated with depressive behavior in OVX rats was revealed, for the first time, in the present study. ESC treatment markedly decreased colon mass index, ulcerative area and total histology score. Moreover, ESC reduced colonic MPO activities and TNF-α levels.

Ovariectomy is one of the most commonly used animal models for induction of depression as well as evaluation of antidepressant activity [19–22]. FST is widely used technique to assess antidepressant effects in rodents [23]. According to a pilot study performed before the experiments, a depressive state has been confirmed after 2 weeks of ovariectomy (data not shown). In addition, ESC was found to decrease the immobility time in a previous study performed in our labs [13]. Consistently, the current study revealed the ability of ESC to decrease the immobility time and increase the swimming behavior, as compared to normal or colitis + OVX groups. Open field test ruled out the possibility that motor effect might mediate the

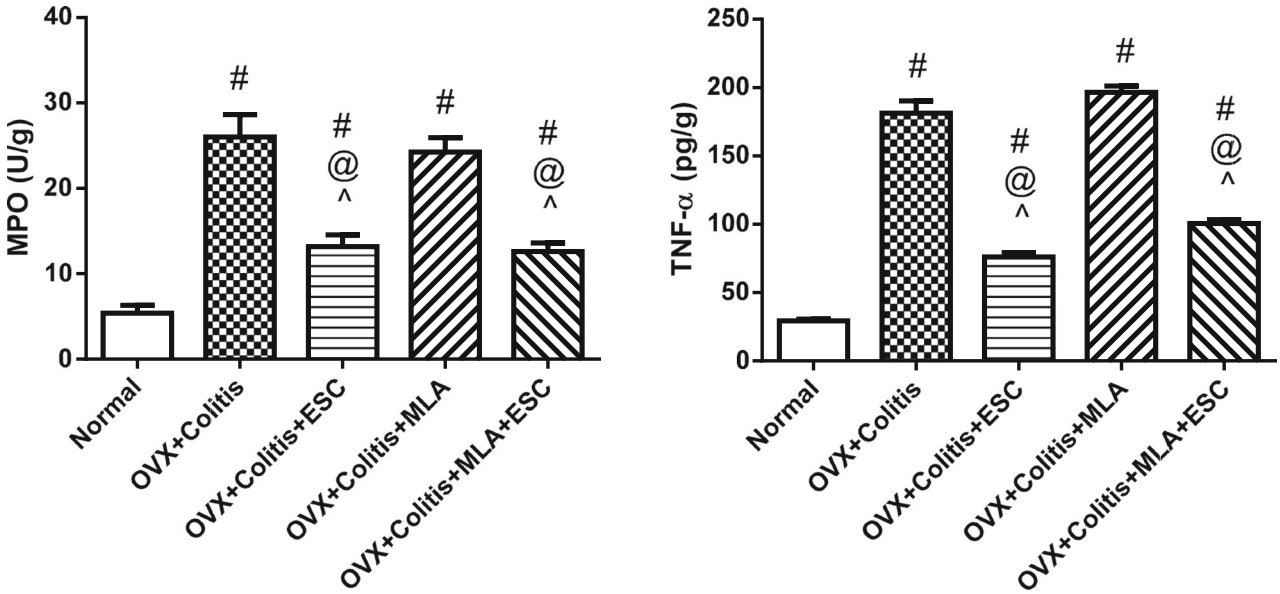



**Fig. 4.** Effect of escitalopram and/or methyllycoconitine (MLA) on **a** myeloperoxidase (MPO) activity, and **b** tumor necrosis factor (TNF)-α in ovariectomized (OVX) rats with iodoacetamide-induced colitis. Data are expressed as means ± SEM of 8 animals. # *vs* normal group, @ *vs* OVX + colitis group, ^ *vs* OVX + colitis+MLA group (one-way ANOVA followed by Tukey-Kramer multiple comparisons test; $p < 0.05$).

antidepressant-like behaviors that observed from ESC treatment.

Iodoacetamide-induced colitis led to a marked loss in body weight as well as increase in colon mass index, ulcerative area, and total histology score, an effect that was previously reported by other authors [12]. This colonic inflammation was associated with massive leukocyte infiltration into the colon as verified by the histological examination as well as measuring colonic MPO activity. Infiltrated leukocytes contribute to disease initiation and subsequent tissue damage through secretion of cytokines such as TNF-α and oxidants [19, 24, 25]. The colonic inflammatory changes induced by iodoacetamide were markedly worsened by OVX. This could be attributed to the fact that stress, including depression and anxiety, may trigger an exacerbation of IBD and lead to more frequent relapses by inducing pro-inflammatory responses [26, 27]. In the present study, ESC markedly improved body weight, colon mass index, ulcerative area, and MPO activities in animals subjected to iodoacetamide-induced colitis with OVX. ESC, by decreasing colonic MPO activities, was expected to possess beneficial effects on colonic inflammation.

Diamond et al. (2006) [28] have studied the anti-inflammatory and immune-modulatory effects of serotonin reuptake inhibitor (fluoxetine and clomipramine) and norepinephrine reuptake inhibitor (reboxetine and desipramine) on monocyte and T cell-derived cytokine production. The study found that antidepressants suppressed the production of the Th1 cytokine IFN-γ in human blood. Moreover, Dong et al. (2016) [7] reported that ESC treatment significantly attenuated lipopolysaccharide-induced rise in serum TNF-α in rats. ESC also lowered plasma TNF-α after cardiac infarction in rats [29]. The present study revealed, for the first time, that ESC significantly reduced the rise in colonic TNF-α levels in an iodoacetamide-induced model of colitis in OVX rats. The anti-colitic effect of other antidepressants, including doxepin and venlafaxine, was previously reported. Doxepin was found to be effective in reducing macroscopic and microscopic colonic parameters as well as MPO activity and cytokines levels in acetic acid-induced colitis model through blocking histamine, serotonin, alpha1 adrenergic, and muscarinic receptors [30]. Venlafaxine showed an anti-inflammatory effect in acetic acid-induced colitis [23] *via* activating noradrenaline and serotonin receptors [31].

The cholinergic pathway is thought to control gut inflammation in experimental colitis [8, 32, 33]. Acetylcholine, the main neurotransmitter of the vagal nerve, was found to control the functions of immune cells and significantly attenuate the release of pro-inflammatory

cytokines, such as TNF-α, in human macrophages through the activation of α7-nAChRs [10]. Therefore, activation of α7-nAChRs effectively attenuated colitis in several animal models [34, 35]. In the current study, the role of α7-nAChR was investigated using the nicotinic receptor antagonist MLA. Treatment OVX + colitis rats with MLA led to marked increase in colonic ulceration, as compared to OVX + colitis control group. This was in agreement with the previous studies of Ghia et al. who revealed that chemical antagonism of α7-nAChR as well as the surgical blockade of vagus nerve significantly worsened the colitis and enhanced the production of colonic inflammatory mediators [8, 33]. ESC administration was found to ameliorate the iodoacetamide-induced colitis in OVX rats even in presence of MLA, indicating that the anti-inflammatory action of ESC is not mainly mediated through α7-nAChR.

In conclusion, the present study revealed the beneficial effect of ESC in iodoacetamide-induced colitis in OVX rats. ESC attenuated the colonic damage and reduced MPO activities and TNF-α levels. Therefore, the present study suggests that ESC may represent a new therapeutic agent for the treatment of IBD, especially in patient with or at high risk of depressive behavior.

## ACKNOWLEDGMENTS

The authors are grateful to Prof. Laila A. Rashed (Medical Biochemistry Department, Faculty of Medicine, Cairo University, Egypt), for her valuable assistance in ELIZA and Western blotting procedures. The authors are also thankful to Prof. Nermeen Shaffie (Department of Pathology, National Research Centre, Cairo, Egypt) for her precious efforts in histological examinations.

## AUTHOR CONTRIBUTIONS

All authors contribute equally.

## FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## COMPLIANCE WITH ETHICAL STANDARDS

**Conflict of Interest.** The authors declare that there is no conflict of interests regarding the publication of this paper.

## REFERENCES

1. Keethy, D., C. Mrakotsky, and E. Szigethy. 2014. Pediatric IBD and depression: Treatment implications. *Current Opinion in Pediatrics* 26 (5): 561–567.
2. Macer, B.J., S.L. Prady, and A. Mikocka-Walus. 2017. Antidepressants in inflammatory bowel disease: A systematic review. *Inflammatory Bowel Diseases* 23 (4): 534–550.
3. Kristensen, M.S., et al. 2018. The influence of antidepressants on the disease course among patients with Crohn's disease and ulcerative colitis—A Danish Nationwide register–based cohort study. *Inflammatory Bowel Diseases* 25 (5): 886–893.
4. Filipovic, B.R., and B.F. Filipovic. 2014. Psychiatric comorbidity in the treatment of patients with inflammatory bowel disease. *World journal of gastroenterology: WJG* 20 (13): 3552.
5. Mikocka-Walus, A.A., A.L. Gordon, B.J. Stewart, and J.M. Andrews. 2012. The role of antidepressants in the management of inflammatory bowel disease (IBD): A short report on a clinical case-note audit. *Journal of Psychosomatic Research* 72 (2): 165–167.
6. Itatsu, T., A. Nagahara, M. Hojo, A. Miyazaki, T. Murai, M. Nakajima, and S. Watanabe. 2011. Use of selective serotonin reuptake inhibitors and upper gastrointestinal disease. *Internal Medicine* 50 (7): 713–717.
7. Dong, C., J.C. Zhang, W. Yao, Q. Ren, C. Yang, M. Ma, M. Han, R. Saito, and K. Hashimoto. 2016. Effects of escitalopram, R-citalopram, and reboxetine on serum levels of tumor necrosis factor-α, interleukin-10, and depression-like behavior in mice after lipopolysaccharide administration. *Pharmacology Biochemistry and Behavior* 144: 7–12.
8. Ghia, J.E., P. Blennerhassett, H. Kumar–Ondiveeran, E.F. Verdu, and S.M. Collins. 2006. The vagus nerve: A tonic inhibitory influence associated with inflammatory bowel disease in a murine model. *Gastroenterology* 131 (4): 1122–1130.
9. Ghia, J.-E., P. Blennerhassett, and S.M. Collins. 2008. Impaired parasympathetic function increases susceptibility to inflammatory bowel disease in a mouse model of depression. *The Journal of Clinical Investigation* 118 (6): 2209–2218.
10. Borovikova, L.V., S. Ivanova, M. Zhang, H. Yang, G.I. Botchkina, L.R. Watkins, H. Wang, N. Abumrad, J.W. Eaton, and K.J. Tracey. 2000. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405 (6785): 458–462.
11. Grandi, A., I. Zini, L. Flammini, A.M. Cantoni, V. Vivo, V. Ballabeni, E. Barocelli, and S. Bertoni. 2017. alpha7 nicotinic agonist AR-R17779 protects mice against 2,4,6-Trinitrobenzene sulfonic acid-induced colitis in a spleen-dependent way. *Frontiers in Pharmacology* 8: 809.
12. Salem, H.A., and W. Wadie. 2017. Effect of niacin on inflammation and angiogenesis in a murine model of ulcerative colitis. *Scientific Reports* 7 (1): 7139.
13. Ibrahim, W.W., M.M. Safar, M.M. Khattab, and A.M. Agha. 2016. 17beta-estradiol augments antidepressant efficacy of escitalopram in ovariectomized rats: Neuroprotective and serotonin reuptake transporter modulatory effects. *Psychoneuroendocrinology* 74: 240–250.
14. Wazea, S.A., W. Wadie, A.K. Bahgat, and H.S. el-Abhar. 2018. Galantamine anti-colitic effect: Role of alpha-7 nicotinic acetylcholine receptor in modulating Jak/STAT3, NF-kappaB/HMGB1/RAGE and p-AKT/Bcl-2 pathways. *Scientific Reports* 8 (1): 5110.

15. Rocha, B.A., R. Fleischer, J.M. Schaeffer, S.P. Rohrer, and G.J. Hickey. 2005. 17β-estradiol-induced antidepressant-like effect in the forced swim test is absent in estrogen receptor-β knockout (BERKO) mice. *Psychopharmacology* 179 (3): 637–643.
16. Estrada-Camarena, E., A. Fernández-Guasti, and C. López-Rubalcava. 2003. Antidepressant-like effect of different estrogenic compounds in the forced swimming test. *Neuropsychopharmacology* 28 (5): 830–838.
17. Wang, W.P., et al. 2001. Protective role of heme oxygenase-1 on trinitrobenzene sulfonic acid-induced colitis in rats. *American Journal of Physiology. Gastrointestinal and Liver Physiology* 281 (2): G586–G594.
18. Bradley, P.P., D.A. Priebat, R.D. Christensen, and G. Rothstein. 1982. Measurement of cutaneous inflammation: Estimation of neutrophil content with an enzyme marker. *The Journal of Investigative Dermatology* 78 (3): 206–209.
19. Abreu, M.T. 2002. The pathogenesis of inflammatory bowel disease: Translational implications for clinicians. *Current Gastroenterology Reports* 4 (6): 481–489.
20. Cryan, J.F., R.J. Valentino, and I. Lucki. 2005. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neuroscience and Biobehavioral Reviews* 29 (4–5): 547–569.
21. Rygula, R., N. Abumaria, G. Flügge, E. Fuchs, E. Rüther, and U. Havemann-Reinecke. 2005. Anhedonia and motivational deficits in rats: Impact of chronic social stress. *Behavioural Brain Research* 162 (1): 127–134.
22. Bogdanova, O.V., S. Kanekar, K.E. D'Anci, and P.F. Renshaw. 2013. Factors influencing behavior in the forced swim test. *Physiology & Behavior* 118: 227–239.
23. Minaiyan, M., V. Hajhashemi, M. Rabbani, E. Fattahian, and P. Mahzouni. 2015. Effect of venlafaxine on experimental colitis in normal and reserpinised depressed rats. *Research in Pharmaceutical Sciences* 10 (4): 295–306.
24. Toms, C., and F. Powrie. 2001. Control of intestinal inflammation by regulatory T cells. *Microbes and Infection* 3 (11): 929–935.
25. Polverini, P. 1997. *Role of the macrophage in angiogenesis-dependent diseases, in Regulation of angiogenesis*, 11–28. Berlin: Springer.
26. Mawdsley, J.E., M.G. Macey, R.M. Feakins, L. Langmead, and D.S. Rampton. 2006. The effect of acute psychologic stress on systemic and rectal mucosal measures of inflammation in ulcerative colitis. *Gastroenterology* 131 (2): 410–419.
27. Mittermaier, C., C. Dejaco, T. Waldhoer, A. Oefferlbauer-Ernst, W. Miehsler, M. Beier, W. Tillinger, A. Gangl, and G. Moser. 2004. Impact of depressive mood on relapse in patients with inflammatory bowel disease: A prospective 18-month follow-up study. *Psychosomatic Medicine* 66 (1): 79–84.
28. Diamond, M., J.P. Kelly, and T.J. Connor. 2006. Antidepressants suppress production of the Th1 cytokine interferon-γ, independent of monoamine transporter blockade. *European Neuropsychopharmacology* 16 (7): 481–490.
29. Bah, T.M., M. Benderdour, S. Kaloustian, R. Karam, G. Rousseau, and R. Godbout. 2011. Escitalopram reduces circulating pro-inflammatory cytokines and improves depressive behavior without affecting sleep in a rat model of post-cardiac infarct depression. *Behavioural Brain Research* 225 (1): 243–251.
30. Zabihi, M., V. Hajhashemi, A. Talebi, and M. Minaiyan. 2017. Evaluation of central and peripheral effects of doxepin on acetic acid-induced colitis in rat and the involved mechanisms. *EXCLI Journal* 16: 414–425.
31. Hashioka, S., P. McGeer, A. Monji, and S. Kanba. 2009. Anti-inflammatory effects of antidepressants: Possibilities for preventives against Alzheimer's disease. *Central Nervous System Agents in Medicinal Chemistry* 9 (1): 12–19.
32. Ghia, J.-E., P. Blennerhassett, R.T. el-Sharkawy, and S.M. Collins. 2007. The protective effect of the vagus nerve in a murine model of chronic relapsing colitis. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 293 (4): G711–G718.
33. Bai, A., Y. Guo, and N. Lu. 2007. The effect of the cholinergic anti-inflammatory pathway on experimental colitis. *Scandinavian Journal of Immunology* 66 (5): 538–545.
34. Wang, H., et al. 2003. Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. *Nature* 421 (6921): 384.
35. Ji, H., M.F. Rabbi, B. Labis, V.A. Pavlov, K.J. Tracey, and J.E. Ghia. 2014. Central cholinergic activation of a vagus nerve-to-spleen circuit alleviates experimental colitis. *Mucosal Immunology* 7 (2): 335–347.