ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Biochemical, Immunohistochemical and Histopathological Studies on the Effect of Probiotic and N- Acetylcysteine on Experimental Ulcerative Colitis.

Alshaimaa M. Said[1]*, Ahmed I. Abo-Ahmed[2], and Shimaa A.E. Atwa[3].

[1]Biochemistry Department, Faculty of Veterinary Medicine, Benha University, Egypt.
[2]Anatomy and Embryology Department, Faculty of Veterinary Medicine, Benha University, Egypt.
[3]Educational Hospital, Faculty of Veterinary Medicine, Benha University, Egypt.

ABSTRACT

The present study was carried out to explore the protective effects of probiotic and N-acetylcysteine (NAC) on acetic acid induced ulcerative colitis. Thirty two adult albino rats were divided into four equal groups; control group, ulcerative colitis group, Probiotic group and NAC group administered (1.62 g/kg) probiotic and (108 mg/kg) NAC respectively for 18 days. After one week of the experiment the ulcerative colitis, Probiotic and NAC groups received intracolonic injection of 2 mL acetic acid 4% for four days for induction of ulcerative colitis. Probiotic and NAC administration significantly decreased the activity of colonic myeloperoxidase (MPO), and down-regulated the inflammatory markers gene expression which were up-regulated by acetic acid. In addition, colonic catalase (CAT) and superoxide dismutase (SOD) activities and glutathione (GSH) concentration were increased by probiotic and markedly increased by NAC comparing to ulcerative colitis group while malondialdehyde (MDA) concentration decreased. Moreover, Probiotic and NAC exhibited protective action against the immunohistochemical and histopathological changes induced by acetic acid in rat colon. In a conclusion, probiotic and NAC had significant ameliorating effects against acetic acid induced ulcerative colitis possibly attributed to their anti-inflammatory and antioxidant properties.

Keywords: Acetic acid, anti-inflammatory, antioxidant, N-acetylcysteine, probiotic, ulcerative colitis.

https://doi.org/10.33887/rjpbcs/2019.10.3.5

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Inflammatory bowel diseases (IBD) are a group of chronic inflammatory disorders of the gastrointestinal tract including Crohn's disease (transmural intermittent inflammation and may involve any part of the gastrointestinal tract) and ulcerative colitis (continuous inflammation, limited to the mucosa and confined to the colon). They have been a worldwide health care problem with a continually increasing incidence, posing a significant morbidity and poor quality of patient life [1]. The etiology of IBD is multifactorial, including genetic susceptibility, bacterial translocation through the injured mucosa and immunological, oxidative stress and environmental factors contributed in both initiation and progression of the disease [2]. Clinical symptoms involve diarrhea, blood in the stool, abdominal pain, weight loss, fatigue and extra-intestinal manifestations as primary sclerosing cholangitis, skin lesions or joint problems [3]. Currently, there is no curative treatment and most IBD patients need long life treatment [4] therefor, new therapeutic alternatives, especially dietary supplements with antioxidant properties, are suggested to improve the quality of patient life.

Probiotic is a live organism that exerts beneficial effects to the host when consumed in adequate quantities. Lactobacilli, bifidobacteria and nonpathogenic yeasts such as Saccharomyces boulardii are the most commonly used probiotics. They could alleviate lactose intolerance, decrease duration of gastrointestinal infections, suppress cancer, reduce plasma cholesterol and improve digestion and nutritional value of foods [5].

N-acetylcysteine (NAC), a small molecule antioxidant containing thiol (SH) group, is widely used in clinical practice as a mucolytic drug [6] and in treatment of pulmonary oxygen toxicity, paracetamol toxicity, immunodeficiency viral infection, lung and liver ischaemic damage and toxicity, and experimental ischaemic skin flaps [7]. It has an anti-inflammatory effect in cerebral and pulmonary inflammation [8]. Also, it has a potent antioxidant effect through scavenging reactive oxygen species and raising the intracellular cysteine and glutathione concentrations. It restores glutathione depleted by drug conjugation in liver tissue [9].

MATERIAL AND METHODS

Animal model

Thirty two adult male Wistar albino rats weighing 200-250 g obtained from the animal house of Faculty of Veterinary Medicine, Benha University, Egypt. They were acclimatized to the laboratory conditions for two weeks before starting the experiment with free access to standard diet and water. Animal handling procedures were carried out according to the guidelines of Faculty of Veterinary Medicine, Benha University Ethical Committee.

Chemicals and Drugs

Acetic acid (4% concentration, ready-made) was obtained from VACSERA, Egypt. Probiotic was supplied by El-Nasr pharmaceutical chemicals, Egypt. NAC was supplied by SEDICO pharmaceutical Co, Egypt.

Animals grouping

Rats were randomly and equally divided into four groups (eight animals each).

- Group 1 administered saline for 18 days and used as control group.
- Group 2, 3, 4 were subjected in the 8th day to the induction of ulcerative colitis by intracolonic injection of 2 mL acetic acid 4% for four days [10].
- Group 3 (Probiotic group): administered probiotic (1.62 g/kg, oral) and Group 4 (NAC group): administered NAC (108 mg/kg, oral) for 18 days [11].

Induction of ulcerative colitis

All animals (except control group) were fasted for 6 hours, with access to water ad libitum and anesthetized by an intraperitoneal injection of 1% sodium pentobarbital in a dose of 50 mg/kg before induction of colitis. Acetic acid 4% infused for 30 sec using a soft pediatric catheter (6 F in size and 2 mm in

diameter), inserted through the rectum into the colon up to a distance of 8 cm and maintained in a supine trendelenburg position for 30 sec to prevent leakage of the intracolonic instill.

Handling of tissue samples

On the 18th day of the experiment, colon were collected after sacrificing the animals, rinsed in ice cold saline and divided into three parts. The first part used for biochemical analysis after homogenization, centrifugation and separation of the supernatant. The second part kept at -80 °C until used for Quantitative RT-PCR. The third part placed in 10% formalin solution for histopathological and immunohistochemical evaluation.

Assessment of inflammatory markers

1. Myeloperoxidase (MPO) activity in colon tissue was measured according to Kettle and Winterbourn [12].
2. Estimation of IL-1β and TNF-α gene expression by RT-PCR:

Total RNA was isolated from rat colon using Gene JET RNA Purification Kit (Thermo Scientific, # K0731, USA) according to the manufacturer's protocol. The complementary DNA was synthesized by RevertAid H Minus Reverse Transcriptase (Thermo Scientific, #EP0451, USA) using 5 µg of total RNA. The primer sequences used for amplification summarized in (Table 1).

Table 1: Forward and reverse primers sequence for qRT-PCR.

Genes	Primers sequences
B-actin	**F:** ACCCACACTGTGCCCATCTA **R:** CGTCACACTTCATGATG
IL-1β	**F:** CACCTCTCAAGCAGAGCACAG **R:** GGGTTCCATGGTGAAGTCAAC
TNF-α	**F:** GCATGATCCGCGACGTGGAA **R:** AGATCCATGCCGTTGGCCAG

Quantitative RT-PCR for IL-1β and TNF-α genes were performed using StepOnePlus RT-PCR system (Applied Biosystem, USA) with initial denaturation at 95 °C for 10 min, 40-45 amplification cycles at 95 °C for 15 sec, annealing at 60 °C for 30 sec and extension at 72 °C for 30 sec. At the end of the last cycle, the temperature was increased from 63 to 95 °C for melting curve analysis. The cycle threshold (Ct) values were calculated for target genes and the housekeeping gene (β actin), and relative gene expression was determined using $2^{-\Delta\Delta Ct}$ method [13].

Assessments of oxidative stress parameters

The oxidative status was assessed by determining CAT [14] and SOD [15] activities, MDA level [16] and GSH [17] concentration using commercially available kits (Laboratory Biodiagnostics Co. Cairo, Egypt).

Histological and immunohistochemical analysis

Parts of the removed rat colon were fixed in 10% buffered formalin, embedded into paraffin blocks, processed to obtain sections with 5 µm thickness, stained with hematoxylin and eosin (H&E) and examined with a light microscope.

For immunohistochemical evaluation, paraffin embedded colon sections were de-paraffinized (in xylene), rehydrated in alcohol and incubated in 3% hydrogen peroxide (H_2O_2) in methanol to block endogenous peroxidase activity. Antigen unmasking performed by incubation in citrate buffer (pH 6.0) then 5% normal goat

serum in phosphate buffered saline. Later, sections were incubated with primary antibodies against inducible nitric oxide synthase (iNOS) and proliferating cell nuclear antigen (PCNA), followed by specific biotin-conjugated secondary antibodies (Neo Markers/Lab vision, Fremont, California, USA). For color development, streptavidin-peroxidase kits were added to the slides and sections were counterstained with Mayer's hematoxylin. For quantification, positively stained cells (brown-colored) were identified using an image analyzer computer system.

Statistical analysis

Results were expressed as mean ± S.E.M. and analyzed by one way analysis of variance (ANOVA) followed by Duncan test using SPSS for windows (Version 18). All values at P ≤0.05 were considered to be significant.

RESULTS

Effect of probiotic and NAC on the inflammatory and oxidative stress markers in colon tissue of rats with ulcerative colitis

Intracolonic injection of acetic acid significantly increased colon MPO activity, up-regulated IL-1β and TNF-α gene expression. Additionally, colon CAT and SOD activities and GSH concentration significantly decreased while concentration of MDA increased. However, Co-administration of probiotic or NAC decreased MPO activity, down-regulated IL-1β and TNF-α gene expression, increased antioxidant enzyme activities and GSH concentration and decreased MDA concentration comparing to ulcerative colitis group. Interestingly, NAC administration significantly improved the inflammatory and oxidative stress effects in comparison with Probiotic group as shown in table (2).

Table 2: Effect of Probiotic and NAC on the inflammatory and oxidative stress markers in colon tissue of rats with ulcerative colitis

	MPO (U/mg)	IL-1β	TNF-α	CAT (µmol/g)	SOD (U/g)	GSH (ng/g)	MDA (µmol/g)
Group 1	0.07 ± 0.01^{d}	1.00 ± 0.07^{d}	1.00 ± 0.05^{d}	55.68 ± 2.05^{a}	77.04 ± 1.35^{a}	5.23 ± 0.20^{a}	18.11 ± 1.63^{d}
Group 2	0.63 ± 0.04^{a}	6.41 ± 0.38^{a}	4.59 ± 0.26^{a}	15.44 ± 2.56^{d}	21.47 ± 2.10^{d}	0.92 ± 0.05^{d}	62.26 ± 2.34^{a}
Group 3	0.32 ± 0.03^{b}	3.25 ± 0.19^{b}	2.93 ± 0.14^{b}	25.73 ± 2.38^{c}	43.80 ± 1.88^{c}	1.82 ± 0.14^{c}	36.15 ± 1.49^{b}
Group 4	0.19 ± 0.02^{c}	1.87 ± 0.11^{c}	1.71 ± 0.10^{c}	36.79 ± 1.49^{b}	64.01 ± 2.32^{b}	3.10 ± 0.18^{b}	22.49 ± 1.80^{c}

Group 1: Control group, Group 2: Ulcerative colitis group, Group 3: Probiotic group, Group 4: NAC group. Mean values with different superscript letters in the same column are significantly different at (P ≤0.05).

Immunohistochemical results

Acetic acid dramatically up-regulated the iNOS immunoexpression in colon tissue of rats with experimentally induced ulcerative colitis (Figure 1B). In probiotic and NAC groups, there was moderate (Figure 1C) and weak (Figure 1D) up-regulation of iNOS expression compared to the control group (Figure 1A). Moreover, Acetic acid obviously suppressed the PCNA immunoexpression (Figure 2B) when compared to normal submucosa (Figure 2A). On the other hand, oral administration of probiotic and NAC showed moderate to marked increase in PCNA expression (Figure 2C, D).

ISSN: 0975-8585

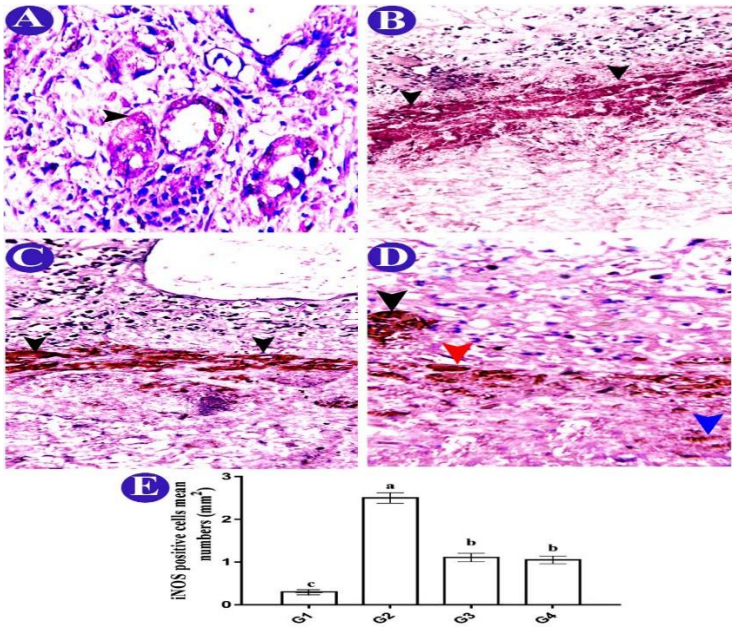

Figure 1: iNOS immunohistochemical expression in colon: A) Control group; B) Ulcerative colitis group: showed very strong positive iNOS staining (arrowheads) in cells located at the junction of crypts and submucosa; C) Probiotic group: showed moderate positive iNOS staining; D) NAC group: showed weak positive iNOS staining (arrowhead), X = 400; E) Quantitative evaluation of iNOS immuno-staining among the four groups.

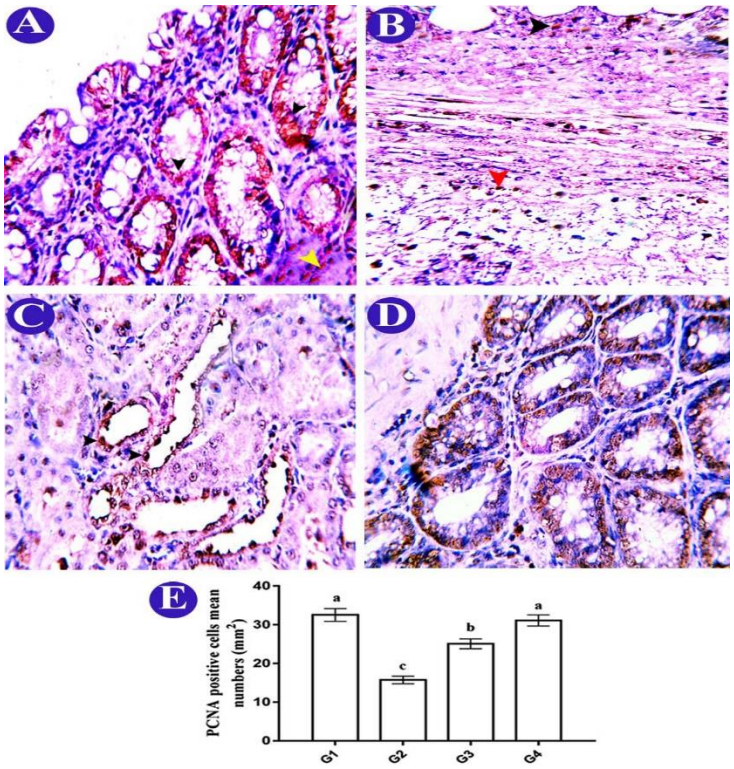

Figure 2: PCNA immunohistochemical expression in colon: A) Control group; B) Ulcerative colitis group: showed very weak positive PCNA staining; C) Probiotic group: showed moderate positive PCNA staining; D) NAC group: showed strong positive PCNA staining, X= 400; E) Quantitative evaluation of PCNA immuno-staining among the four groups.

ISSN: 0975-8585

Macroscopic and microscopic findings

Macroscopic examination revealed severe inflammatory response in the distal colon of ulcerative colitis group represented by hyperemia, edema, ulceration and hemorrhage (Figure $3A_2$) compared to the control group (Figure $3A_1$). However, probiotic moderately attenuated the macroscopic damage and inflammatory response (Figure $3A_3$) while administration of NAC alleviated the colonic damage to an extent close to the normal condition (Figure $3A_4$).

Microscopically, intracolonic injection of acetic acid induced atrophy and/or destruction of intestinal crypts (Figure 3C) as well as submucosal edema revealed by increase in submucosal thickness (Figure 3D) compared to the control group (Figure 3B). Moreover, the mucosa, submucosa and crypts showed inflammatory cellular infiltration (Figure 3C, D, arrowheads). On the contrary, oral administration of probiotic and NAC exhibited regenerative changes except for slight degree of edema in the submucosa and dilated lumen of some crypts (Figure 3E, F).

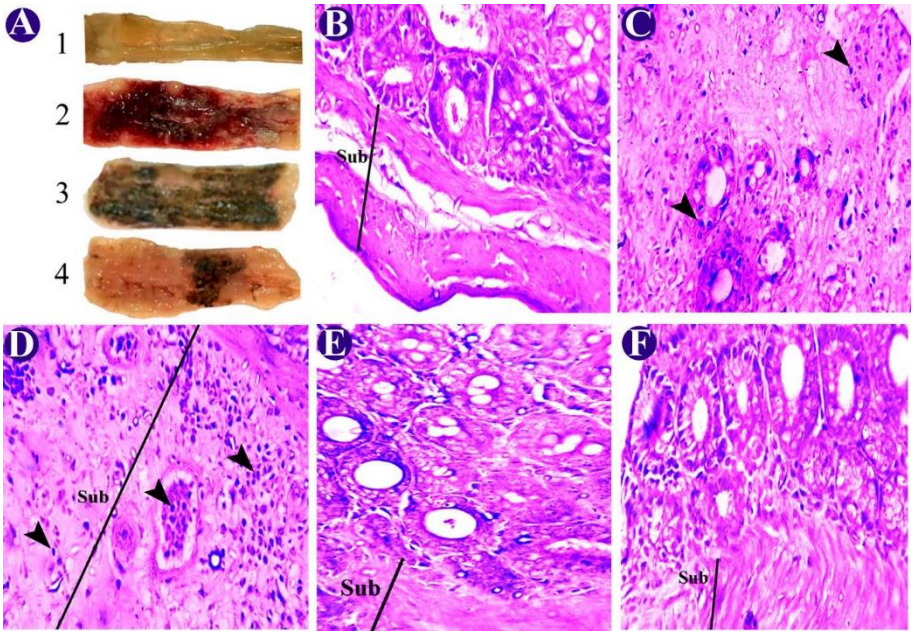

Figure 3: A) Macroscopic appearance of rat colon: 1,2,3,4 refer to control, ulcerative colitis, probiotic and NAC groups respectively. Microscopic examination of rat colon: B) Control group: showing normal crypt structure and submucosa (Sub); C, D) Ulcerative colitis group: showing destruction of some crypts with inflammatory cellular infiltration (arrowheads) and edema of the submucosa; E) Probiotic group; F) NAC group.(H&E stain, X 400).

DISCUSSION

Intestinal inflammation, edema, and ulceration of the colonic mucosa in UC produce cyclic bouts of clinical symptoms including diarrhea, rectal bleeding, and anemia [18]. Acetic acid induced colitis is a standard and popular method to produce an experimental model of UC similar to human in terms of histological features, increased production of reactive oxygen species, ulceration, inflammation and disturbance in mucosal permeability [19].

The current study aimed to explore the effects of probiotic and NAC on acetic acid induced colitis and our results clearly showed that they could inhibit experimental colitis in variable degree. NAC caused a dramatic reduction in the severity of colitis which was comparable to probiotic. This effect is possibly attributed to their anti-inflammatory and antioxidant properties as indicated by correction of the increased inflammatory markers and oxidative stress indices in colon tissue and improved macroscopic and microscopic features.

Exposure to acetic acid in our experiment was accompanied by a profound inflammatory response as shown in (Table 2) which came in agreement with previous studies who reported threefold and sevenfold increase in MPO activity [20, 21]. Also, significant increase in colonic concentration of IL-1β and TNF-α after colitis induction recorded by the authors of [20, 22].

Acetic acid induced colitis characterized by an increase in MPO activity, an indicator of neutrophils infiltration into the colon as shown in ulcerative colitis group [23]. Myeloperoxidase, removes H_2O_2 and catalyzes the formation of toxic hypochlorous acid in the presence of chloride ion which produces reactive oxygen species (ROS) as hydroxyl radicals, singlet oxygen, peroxynitrite, and ozone [24].

Probiotic significantly decreased colonic MPO activity probably through its ability to reduce neutrophil infiltration in the inflamed colon [25]. Also, it is possible that the alteration in gut flora due to probiotic therapy decreases the inflammation, which, in turn, lowers the MPO levels [26]. On the other hand, NAC has been found to be a powerful scavenger of hypochlorous acid (HOCl) which is involved in the inflammatory reaction of colitis and its scavenging may thus account for the observed protective effect of NAC [27]. Therefore, a reduction in the MPO activity can be interpreted as a manifestation of the anti-inflammatory activity of probiotic and NAC.

Ulcerative colitis has been associated with recruitment of inflammatory cells, immunological imbalance and overproduction of ROS and inflammatory cytokines such as IL-1β and TNF-α [28]. These inflammatory cytokines play an important in gastrointestinal immune response [29] however, their overproduction stimulates the secretion of other inflammatory cytokines thereby enhancing and maintaining the inflammatory reaction [30]. It has been reported that, suppressing release of inflammatory cytokines successfully inhibit colitis in animal model [22]. In the current work, there is a significant down-regulation in the expression of inflammatory cytokines in probiotic group when compared to ulcerative colitis group. This could be attributed to the ability of probiotics to regulate the immune response through down-regulation of pro-inflammatory cytokines and up-regulation of anti-inflammatory cytokines as IL-10 [31]. This anti-inflammatory cytokine regulates the TNF-α converting enzyme so it is capable of inhibiting its synthesis [32].

It is important to highlight that, oxidative stress and inflammation are implicated in the pathogenesis of ulcerative colitis [27] so, antioxidants as NAC minimizing ROS and their negative effects [33]. This is consistent with our results (Table 2) and the previous observation of Hsu et al. [34] who noted that, NAC administration to rats with endotoxemia significantly inhibits release of inflammatory cytokines.

The inflammatory reactions observed in ulcerative colitis are associated with nitric oxide (NO) & iNOS, important pro-inflammatory mediators [35], which mediate the increase in thickness of submucosa [36] as seen in (Figure 3C). Also, the significant up-regulation of iNOS immunoexpression associated with dense submucosal inflammatory cell infiltration (Figure 1B) came in accordance with those of Galil and co-authors [37]. The clinical feature of ulcerative colitis is aggravated through the highly toxic peroxynitrite radical, produced from the interaction between NO with superoxide, which activates nuclear factor-κB and increases the iNOS expression [38]. In the present study, administration of probiotic and NAC significantly decreased the iNOS immunoexpression which could be attributed to their antioxidant properties.

Intestinal inflammation is accompanied by ROS overproduction, from the activated phagocytic leukocytes and neutrophils, leading to lipid peroxidation and sever tissue damage [39]. Consistent with this report, we found that, probiotic and NAC supplementation to rats with ulcerative colitis remarkably attenuated the oxidative stress induced by acetic acid.

It was reported that the antioxidant activity of probiotic may include the following mechanisms. 1) Stimulating synthesis of antioxidant enzymes. 2) Stimulating the immune system in order to prevent cytokine induced oxidative stress. 3) Inhibiting intestinal pathogens, to reduce inflammation and associated oxidative damage. 4) Enhancing the absorption of dietary antioxidants [40, 41]. On the other hand, NAC acts directly by scavenging ROS and indirectly by synthesis of glutathione [42, 43].

Intestinal mucosa counteracts various stresses through production of intact physical barrier by its rapidly proliferating cells [2]. Proliferating cell nuclear antigen (PCNA), important index of cell proliferation, is essential in DNA replication and repair [44] and responsible for decisions of life and death of the cell [45]. In

the present work, PCNA proliferation index was suppressed in ulcerative colitis group and up-regulated in groups administered probiotic and NAC. This proliferating effect could be linked to their antioxidant effects and modulating the oxidant/antioxidant balance of the injured colonic mucosa [37].

The macroscopic and microscopic investigations confirm the findings of our biochemical and immunohistochemical results. The observed submucosal edema and inflammatory cellular infiltration indicates sever inflammatory reaction in ulcerative colitis group and goes in parallel with the increased inflammatory markers. In contrast, concomitant treatment with probiotic or NAC significantly ameliorated all the pathological changes and these effects were more marked in the NAC group than in probiotic group. It could be attributed to both strain and dose of probiotic [46] which require further investigation.

CONCLUSION

The results in this study confirm that probiotic and NAC have protective effects on acetic acid induced ulcerative colitis, related to their anti-inflammatory and antioxidant properties. Therefore, these compounds may be candidate agents for the development of natural anticolitis therapy.

Author Contribution

Conceived & designed the experiments: Alshaimaa Said, Performed the experiments: Ahmed Abo-Ahmed and Shimaa Atwa, Analyzed the data: Alshaimaa Said, Immunohistochemical and Histopathological analysis: Ahmed Abo-Ahmed, Wrote the paper: Alshaimaa Said and Ahmed Abo-Ahmed.

Conflict of interest

This research did not receive any specific grant from any funding agency The authors declare that there are no conflicts of interest.

ACKNOWLEDGMENT

The authors would gratefully acknowledge the staff members in the Animal House of Biochemistry Department, Faculty of Veterinary Medicine, Benha University, Egypt for looking after the rats and for technical support in the implementation of this study.

REFERENCES

[1] Molodecky NA, Soon IS, Rabi DM, Ghali WA, Ferris M, Chernoff G, Benchimol EI, Panaccione R, Ghosh S, Barkema HW and others. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 2012; 142(1):46-54.

[2] Abraham C, Cho JH. Inflammatory bowel disease. The New England journal of medicine 2009; 361(21):2066-2078.

[3] Frei P, Biedermann L, Nielsen OH, Rogler G. Use of thiopurines in inflammatory bowel disease. World journal of gastroenterology 2013; 19(7):1040-1048.

[4] Vecchi Brumatti L, Marcuzzi A, Tricarico PM, Zanin V, Girardelli M, Bianco AM. Curcumin and inflammatory bowel disease: potential and limits of innovative treatments. Molecules 2014; 19(12):21127-53.

[5] Shanahan F. Probiotics and inflammatory bowel disease: is there a scientific rationale? Inflamm Bowel Dis 2000; 6(2):107-15.

[6] Tirouvanziam R, Conrad CK, Bottiglieri T, Herzenberg LA, Moss RB, Herzenberg LA. High-dose oral N-acetylcysteine, a glutathione prodrug, modulates inflammation in cystic fibrosis. Proc Natl Acad Sci U S A 2006; 103(12):4628-33.

[7] Rutgeerts P, Van Deventer S, Schreiber S. Review article: the expanding role of biological agents in the treatment of inflammatory bowel disease - focus on selective adhesion molecule inhibition. Aliment Pharmacol Ther 2003; 17(12):1435-50.

[8] Tobwala S, Khayyat A, Fan W, Ercal N. Comparative evaluation of N-acetylcysteine and N-acetylcysteineamide in acetaminophen-induced hepatotoxicity in human hepatoma HepaRG cells. Experimental biology and medicine (Maywood, N.J.) 2015; 240(2):261-272.

[9] Rushworth GF, Megson IL. Existing and potential therapeutic uses for N-acetylcysteine: the need for conversion to intracellular glutathione for antioxidant benefits. Pharmacol Ther 2014; 141(2):150-9.
[10] Fabia R, Willen R, Ar'Rajab A, Andersson R, Ahren B, Bengmark S. Acetic acid-induced colitis in the rat: a reproducible experimental model for acute ulcerative colitis. Eur Surg Res 1992; 24(4):211-25.
[11] Paget GE, Barnes JM. Evaluation of Drug Activities; 1964.
[12] Kettle AJ, Winterbourn CC. Assays for the chlorination activity of myeloperoxidase. Methods Enzymol 1994; 233:502-12.
[13] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001; 25(4):402-408.
[14] Aebi H. Catalase in vitro. Methods Enzymol 1984; 105:121-6.
[15] Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 1984; 21(2):130-132.
[16] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95(2):351-358.
[17] Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979; 582(1):67-78.
[18] Mahmoud B, Shady A, Kifafy M, El-Seify G, Eldamhogy R, Omara R. The effect of royal jelly versus sulfasalazine on acetic acid-induced colitis in adult albino rats. Menoufia Medical Journal 2015; 28(3):748-756.
[19] Ma L, Chow JY, Cho CH. Mechanistic study of adverse actions of cigarette smoke exposure on acetic acid-induced gastric ulceration in rats. Life Sci 1998; 62(3):257-266.
[20] Matuszyk A, Ceranowicz P, Warzecha Z, Cieszkowski J, Bonior J, Jaworek J, Kusnierz-Cabala B, Konturek P, Ambrozy T, Dembinski A. Obestatin Accelerates the Healing of Acetic Acid-Induced Colitis in Rats. Oxid Med Cell Longev 2016; 2016:1-7.
[21] Xing JF, Sun JN, Sun JY, Hu SS, Guo CN, Wang ML, Dong YL. Protective effect of shikimic acid on acetic acid induced colitis in rats. J Med Plants Res. 2012; 6:2011-2018.
[22] Ashry EE, Abdellatief BR, Refaiy A, Kotb IH. Protective Effect of Ketamine against Acetic Acid-Induced Ulcerative Colitis in Rats. Pharmacology & Pharmacy 2016; 7:9-18.
[23] Higa A, Eto T, Nawa Y. Evaluation of the role of neutrophils in the pathogenesis of acetic acid-induced colitis in mice. Scand J Gastroenterol 1997; 32(6):564-568.
[24] Loft S, Poulsen HE. Cancer risk and oxidative DNA damage in man. J Mol Med (Berl) 1996; 74(6):297-312.
[25] Peran L, Sierra S, Comalada M, Lara-Villoslada F, Bailon E, Nieto A, Concha A, Olivares M, Zarzuelo A, Xaus J and others. A comparative study of the preventative effects exerted by two probiotics, Lactobacillus reuteri and Lactobacillus fermentum, in the trinitrobenzenesulfonic acid model of rat colitis. Br J Nutr 2007; 97(1):96-103.
[26] Russo AJ. Decreased plasma myeloperoxidase associated with probiotic therapy in autistic children. Clinical medicine insights. Pediatrics 2015; 9:13-17.
[27] Cetinkaya A, Bulbuloglu E, Kurutas EB, Ciralik H, Kantarceken B, Buyukbese MA. Beneficial effects of N-acetylcysteine on acetic acid-induced colitis in rats. Tohoku J Exp Med 2005; 206(2):131-9.
[28] Bertevello PL, Logullo AF, Nonogaki S, Campos FM, Chiferi V, Alves CC, Torrinhas RS, Gama-Rodrigues JJ, Waitzberg DL. Immunohistochemical assessment of mucosal cytokine profile in acetic acid experimental colitis. Clinics (Sao Paulo) 2005; 60(4):277-86.
[29] Roya H, Mirzaei A. Protective effects of lippia citriodora on acetic acid induced ulcerative colitis in rats World journal of pharmacy and pharmaceutical sciences 2014; 3(10):211-221.
[30] Polk WH, Jr., Dempsey PJ, Russell WE, Brown PI, Beauchamp RD, Barnard JA, Coffey RJ, Jr. Increased production of transforming growth factor alpha following acute gastric injury. Gastroenterology 1992; 102(5):1467-1474.
[31] Karamese M, Aydin H, Sengul E, Gelen V, Sevim C, Ustek D, Karakus E. The Immunostimulatory Effect of Lactic Acid Bacteria in a Rat Model. Iran J Immunol 2016; 13(3):220-228.
[32] Brennan FM, Green P, Amjadi P, Robertshaw HJ, Alvarez-Iglesias M, Takata M. Interleukin-10 regulates TNF-alpha-converting enzyme (TACE/ADAM-17) involving a TIMP-3 dependent and independent mechanism. Eur J Immunol 2008; 38(4):1106-1117.
[33] Dodd S, Dean O, Copolov DL, Malhi GS, Berk M. N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility. Expert Opin Biol Ther 2008; 8(12):1955-1962.
[34] Hsu BG, Lee RP, Yang FL, Harn HJ, Chen HI. Post-treatment with N-acetylcysteine ameliorates endotoxin shock-induced organ damage in conscious rats. Life Sci 2006; 79(21):2010-2016.

[35] Gillberg L, Varsanyi M, Sjostrom M, Lordal M, Lindholm J, Hellstrom PM. Nitric oxide pathway-related gene alterations in inflammatory bowel disease. Scand J Gastroenterol 2012; 47(11):1283-1297.
[36] Schreiber O, Petersson J, Walden T, Ahl D, Sandler S, Phillipson M, Holm L. iNOS-dependent increase in colonic mucus thickness in DSS-colitic rats. PLoS One 2013; 8(8):1-9.
[37] Galil TAE, ElGhamrawy TA, Sadik AOE. The Effect of N. Acetylcysteine and Ginger on Acetic Acid Induced Colitis in Adult Male Albino Rat: Histological, Immunohistochemical and Morphometric Study. J Cytol Histol 2015: 1-8.
[38] Cooke CL, Davidge ST. Peroxynitrite increases iNOS through NF-kappaB and decreases prostacyclin synthase in endothelial cells. Am J Physiol Cell Physiol 2002; 282(2):395-402.
[39] Jena G, Trivedi PP, Sandala B. Oxidative stress in ulcerative colitis: an old concept but a new concern. Free Radic Res 2012; 46(11):1339-1345.
[40] Martarelli D, Verdenelli MC, Scuri S, Cocchioni M, Silvi S, Cecchini C, Pompei P. Effect of a probiotic intake on oxidant and antioxidant parameters in plasma of athletes during intense exercise training. Curr Microbiol 2011; 62(6):1689-1696.
[41] Mikelsaar M, Zilmer M. Lactobacillus fermentum ME-3 - an antimicrobial and antioxidative probiotic. Microbial ecology in health and disease 2009; 21(1):1-27.
[42] Dekhuijzen PNR. Antioxidant properties of N-acetylcysteine: their relevance in relation to chronic obstructive pulmonary disease. European Respiratory Journal 2004; 23(4):629-636.
[43] Sadowska AM, Manuel YKB, De Backer WA. Antioxidant and anti-inflammatory efficacy of NAC in the treatment of COPD: discordant in vitro and in vivo dose-effects: a review. Pulm Pharmacol Ther 2007; 20(1):9-22.
[44] Essers J, Theil AF, Baldeyron C, van Cappellen WA, Houtsmuller AB, Kanaar R, Vermeulen W. Nuclear dynamics of PCNA in DNA replication and repair. Mol Cell Biol 2005; 25(21):9350-9359.
[45] Paunesku T, Mittal S, Protic M, Oryhon J, Korolev SV, Joachimiak A, Woloschak GE. Proliferating cell nuclear antigen (PCNA): ringmaster of the genome. Int J Radiat Biol 2001; 77(10):1007-1021.
[46] Orel R. Inflammatory bowel disease, intestinal microflora, prebiotics and probiotics International Journal of Sanitary Engineering Research 2009;3(1):36-48.