International Journal of Pharmacognosy and Pharmaceutical Sciences
Online ISSN: 2706-7017; Print ISSN: 2706-7009; Impact Factor: RJIF 5.44
Received: 25-11-2020; Accepted: 10-12-2020; Published: 20-12-2020
www.pharmacognosyjournal.net
Volume 2; Issue 1; 2020; Page No. 17-23

Effect of N-acetylcysteine on an experimental model of indomethacin induced inflammatory bowel disease

Yara Annouf[1, 2*], Shaza Al laham[1, 2], Eyad Chatty[3]
[1] Department of Pharmacology and Toxicology Faculty of Pharmacy, Damascus University, Damascus, Syria
[2] Department of Pharmacology and Toxicology Faculty of Pharmacy, Syrian Private University, Damascus, Syria
[3] Department of Pathology Faculty of Medicine, Damascus University, Damascus, Syria
DOI: https://doi.org/10.33545/27067009.2020.v2.i1a.19

Abstract
N-acetylcysteine (NAC) is a thiol compound can act both as a precursor of GSH and as a direct ROS scavenger. Moreover, NAC has been purported to have anti-inflammatory properties. The aim of this study was to investigate the effects of NAC on a rat model of inflammatory bowel disease (IBD). Model of IBD was induced by subcutaneous Indomethacin (Indo) at a dose rate of 9 mg/kg for two days at 24 h intervals. NAC in two doses (500mg/Kg, 1 g/kg body weight po) was administrated for seven consecutive days beginning 24 h after the first Indo injection. Body weight loss, small intestine weight / length ratio, macroscopic damage, histological study, as well as by biochemical measurement of reduced glutathione (GSH) and superoxide dismutase (SOD) activity in the small intestine tissue were used for assessment of small intestinal injury. NAC in two doses especially at dose (1g/kg) revealed increase in small intestine weight/length ratio, macroscopic and microscopic small intestinal damage scores caused by administration of indomethacin with no statistical significance comparing with Indo control group ($p>0.05$). The increased of the oxidative stress was observed in both doses of NAC especially at dose (1g/kg) by decrease the levels of GSH and SOD activity with no statistical significance comparing with Indo control group ($p>0.05$). The conclusion of this study is NAC didn't enhance intestinal inflammation induced by Indo (rat model of IBD) in both doses, in contrast rising in inflammation and oxidative stress was observed in NAC treated groups especially at dose 1g/kg

Keywords: inflammatory bowel disease, indomethacin, N-acetylcysteine, superoxide dismutase, reduced glutathione, lipid peroxides

Introduction
Clinically, inflammatory bowel disease (IBD) is a chronic inflammatory condition of the intestines that is marked by remission and relapses and distills clinically into one of two major subtypes of disease: ulcerative colitis (UC) and Crohn disease (CD) [1]. IBD causes significant gastrointestinal symptoms that include diarrhea, abdominal pain, bleeding, anemia and weight loss [2]. The pathogenesis of IBD is not fully defined, but is clearly multifactorial, resulting from multiple genes, developmental and environmental factors, which together result in a dysregulated innate and adaptive mucosal immune response [3]. In recent years, several studies had focused on reactive oxygen species (ROS) and reactive nitrogen species (RNS) as the etiologic factors for IBD [4]. Accumulating data from both experimental models and clinical studies indicate that oxidative stress signaling is involved in and contributes to the development of IBD through multiple levels of function. Oxidative stress leads to damages of the mucosal layer in the GI tract and bacterial invasion, which in turn stimulates the immune response and initiates IBD [5].
Systemic administration of Indo, a nonsteroidal anti-inflammatory drug, to rats results in inflammation of the small intestine, which has been used extensively as an experimental model of CD [6]. It induced enteritis shares clinical, histological, and pathophysiological characteristics with CD [7]. Several authors reported that the histomorphological picture of Indo induced inflammation has a similar picture to that of CD [8]. Although the mechanisms of Indo-induced rat intestinal ulceration and human IBD may differ, the fundamental inflammatory processes are similar. Therefore, Indo-induced intestinal ulceration may provide a useful tool to better understand the pathogenesis of IBD and to search for effective therapies [9].
N-acetylcysteine (NAC) is a precursor to the amino acid L-cysteine and consequently the antioxidant glutathione (GSH). It has been used for more than 50 years, there are still many controversies surrounding it as a medicine as well as a dietary supplement [10]. NAC is being studied and utilized in conditions characterized by decreased GSH or oxidative stress such as HIV infection, cancer, and heart disease. Because of its hepato-protective activity, intravenous and oral administrations of NAC have been used extensively in the management of acetaminophen poisoning [11]. Moreover, NAC has been purported to have anti-inflammatory properties. Induction of the pro-inflammatory transcription factors activator protein1 (AP-1) and NF-κB is inhibited by NAC [12].
The present study was planned to test the effect of N-acetylcysteine on the rat model of IBD induced by Indo.

Materials and methods
Animal and experimental design
Female and male wistar albino rats weighing 160-290 g were purchased from the Scientific Research Center, Damascus, Syria. The animals were provided with ad libitum feed and water. The animals were kept at controlled environmental conditions (temperature 23 ± 2°C, humidity 55 ± 15%, lighting regimen of 12h light:12-h dark). They were acclimatized for one week before any experimental.

All methods in this study were performed in concordance with regulatory guidelines on the care and use of laboratory animals; National Research council [NRS] 2011. Guide for the Care and Use of Laboratory Animals. 8th Washington: National Academies Press.

Animals were randomly divided into four groups:

Group I: normal control group (6 rats in this group) received oral vehicle (physiological saline).

Group II: Indo control group (7 rats in this group) received subcutaneous Indo prepared in 5 % sodium bicarbonate, administered at a dose rate of 9 mg/kg for two days at 24h intervals. It also received oral vehicle (physiological saline).

Group III: NAC treated group (8 rats in this group) received NAC dissolved in physiological saline (500 mg/kg body weight po) for seven consecutive days beginning 24 h after the first Indo injection.

Group IV: NAC treated group (6 rats in this group) received NAC dissolved in physiological saline (1 g/kg body weight po) for seven consecutive days beginning 24h after the first Indo injection. Three and four groups were given subcutaneous Indo prepared in 5 % sodium bicarbonate and administered at a dose rate of 9 mg/kg for two days at 24h intervals. On day eight, each sub group of animals across all groups was sacrificed. The small intestine was removed and was opened longitudinally along their anti-mesenteric borders, tissues were washed in saline solution, and any macroscopic change was checked. A precise evaluation of the lesions was made after each specimen was fixed in 10% formalin.

Intestinal tissue from jejunum was collected and stored at -80 °C till further analysis.

Clinical findings

During the study, rats were checked daily for body weight, behavioral changes, food intake, intestinal bleeding and stool consistency. The body weight of animals was measured at regular time intervals from day 0 to 7 and change of body weight (%) was calculated.

Small intestine weight/length ratio

The length and weight of the small intestine was measured for the estimation of:

Weight of the intestine (g)/length of the intestine (cm) ratio

Macroscopic characters [13]

Table 1: Macroscopic inflammation assessment of the small intestine

Score	Macroscopic score
0	No visible change
1	Hyperemia at sites
2	Lesions having diameter l mm or less
3	Lesions having diameter 2 mm or less (number < 5)
4	Lesions having diameter 2 mm or less (number 5–10)
5	Lesions having diameter 2 mm or less (number > 10)
6	Lesions having diameter more than 2 mm (number < 5)
7	Lesions having diameter more than 2 mm (number 5–10)
8	Lesions having diameter more than 2 mm (number > 10)

Histopathological observations

A portion of the distal small intestine (jejunum) specimen from each rat was fixed with 10% formalin, embedded in paraffin wax and cut into sections of 5mm thickness. The sections were stained with hematoxylin and eosin (H and E) dye for histopathological observations. The following histological features were examined by an unbiased pathologist (AM) blinded to the experimental design: grade and type of inflammation, extension of inflammation throughout the gastrointestinal wall (mucosa, submucosa, muscular layer and serous membrane), presence of Lymphocytic Follicle/aggregate, Necrosis, Granuloma, Cryptitis, Crypt abscess and epithelial lesions (erosions, ulcers) [14].

Biochemical estimations

Accurately weighed tissues from jejunum were homogenized in cold phosphate buffered saline [pH 7.4, 50 mM] to prepare 10 % homogenate and the suspension was divided into three portions. One part of tissue suspension was mixed with 0.2 ml 5 % trichloroacetic acid for measurement of GSH levels, second part of tissue suspension was used for measurement SOD activity. One and two parts of tissue homogenate were centrifuged at 10000g for 20 min at 4º C and supernatant was used for assay GSH levels and SOD activity.

Assay of reduced glutathione (GSH)

Reduced glutathione (GSH) was measured by reaction with 5, 5'-dithiobis (2- nitrobenzoic acid) (DTNB) to give a compound that absorbs at 412nm (Ellman's method). In short, each sample cuvette contained 2ml 0.6mM DTNB in 0.2M sodium phosphate, pH 8.0, 0.1-0.2ml supernatant fraction, and 0.2M phosphate buffer to a final volume of 3ml. (Measurement of the pH in the cuvette showed that the buffer capacity was sufficient to neutralize the trichloroacetic acid present in the sample, and assay of known amounts of GSH in the presence of 0.1-0.2ml 5% trichloroacetic acid demonstrated that this substance did not interfere with the procedure in any other way.) The reference cuvette contained 0.1-0.2ml 5% trichloroacetic acid instead of sample, and the reaction was started by the addition of supernatant to the sample cuvette [15]. It is expressed as µM of GSH per gram of tissue.

Assay of superoxide dismutase (SOD) activity

The recommended procedure is as follows. First, a certain amount of pyrogallol solution (60 mM in 1 mM HCl, 37 °C) was thoroughly mixed with pH 7.4 Tris-HCl buffer (0.05 M, 37 °C) containing 1 mM Na2EDTA (to remove metal ions, which may catalyze the reaction); the total volume was adjusted to 3000 µL using the buffer. The A325 nm value of the mixture without a sample was measured every 30 s for 5 min at 37 °C. Second, an amount of pyrogallol solution equal to that used in the first step was added to a mixture with a sample, and the total volume was adjusted to 3000 µL using the buffer.

Enzyme activity which corresponds to amount of enzyme that inhibits auto-oxidation of pyrogallol by 50 % was calculated and expressed per mg of protein [16].

Statistical analysis

Data analyses were achieved using a software program Graph Pad Prism version 8. Data were expressed as mean ± SEM, and different groups were compared using one way analysis of variance (ANOVA) followed by Sidak test for multiple comparisons for parametric data, and Kruskal–Wallis test followed by Dunn test for multiple comparisons for non-parametric data and parametric data that have

shown non normal distribution. P values less than 0.05 were considered statistically significant.

Results

Clinical findings, general observation and body weight change

After 24 h of administration first dose of Indo, animals developed soft feces, weakness, decreased food intake and progressively body weight loss. All these symptoms reached a maximum at three days from first dose of Indo, and then these symptoms started to decrease gradually.

Groups treated with NAC didn't reveal any progress of these symptoms, some rats of these groups revealed diarrhea and intestinal bleeding.

Compared with that of the normal control group which revealed increase in body weight (1.63 %), the body weight of the Indo control group at the end of experiment was reduced by (-4.58%) with statistical significance comparing with normal control group (p=0.0402).

NAC treated group (500 mg/kg) revealed decrease in body weight (-4.89 %) with no statistical significance comparing with Indo control group (p=0.9119).

NAC treated group (1g/kg) revealed decrease in body weight (-12.02%) with statistical significance comparing with Indo control group (p=0.0153) (Table 2).

Table 2: the effects of NAC on the body weight in Indo induced inflammatory bowel disease model in rats

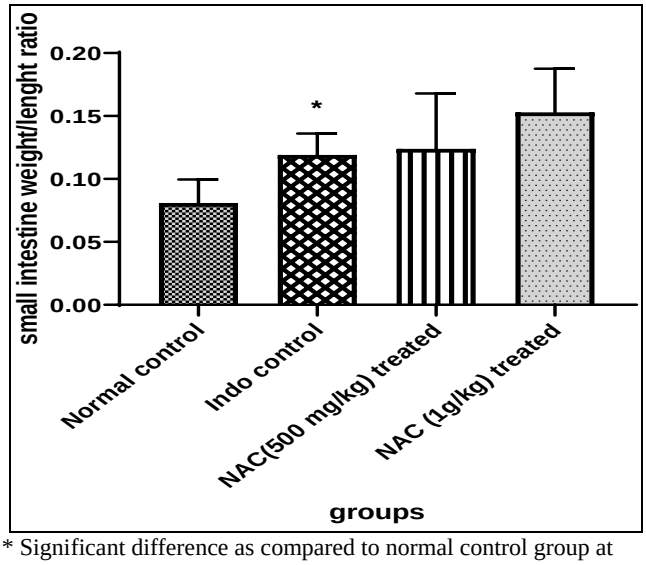
Parameter Group	Initial body weight	Final body weight	Body weight change %
Normal control	194.2 ± 8.29	196.8 ± 7.78	1.63 ± 2.7
Indo control	204.1 ± 13.14	194.9 ± 14.03	-4.58 ± 2.28*
NAC treated (500 mg/kg)	212.5 ± 11.52	202.9 ± 13.68	-4.89 ± 1.46
NAC treated (1g /kg)	221 ± 10.33	193.8 ± 7.12	-12.02 ± 1.74#

Values are given as mean± S.E.M. values are statistically significant at *P<0.05 between normal and Indo control groups, #p<0.05 between NAC treated group (1g /kg) and Indo control groups

Small intestine weight / length ratio

Small intestine weight / length ratio is indirect reliable marker of the small intestine inflammation. The increase in this ratio in Indo control group was observed, there was statistical significance comparing with normal control group (p=0.0372).

NAC treated group (500 mg/kg) revealed increase in small intestine weight / length ratio with no statistical significance comparing with Indo control group (p=0.7667). In addition NAC treated group (1g/kg) revealed increase in small intestine weight/length ratio with no statistical significance comparing with Indo control group (p=0.0606) (Figure 1).



* Significant difference as compared to normal control group at p<0.05

Fig 1: The effects of N-acetylcysteine on small intestine weight / length ratio in Indo induced inflammatory bowel disease model in rats. Data are expressed as mean ±S.E.M

Macroscopic score

The most sections of the distal small intestine in normal control group didn't reveal any morphological changes.

In contrast subcutaneous injection of Indo produced damage in the distal small intestine. Adhesions, erosion, edema, hemorrhagic spots were noticed. These lesions have diameter greater than 2mm, thus the morphological score in the Indo control group was significantly increased (p=0.0006) as compared to normal control group.

Administration of NAC in both doses didn't reveal reduction in the severity of the gross lesion, on the contrary they increased it especially at dose 1g/kg (Figure 2) (Table 3) with no statistical significance comparing with Indo control group [NAC (500mg/kg): p=0.7669, NAC (1g/kg): p= 0.3581] (Figure 3).

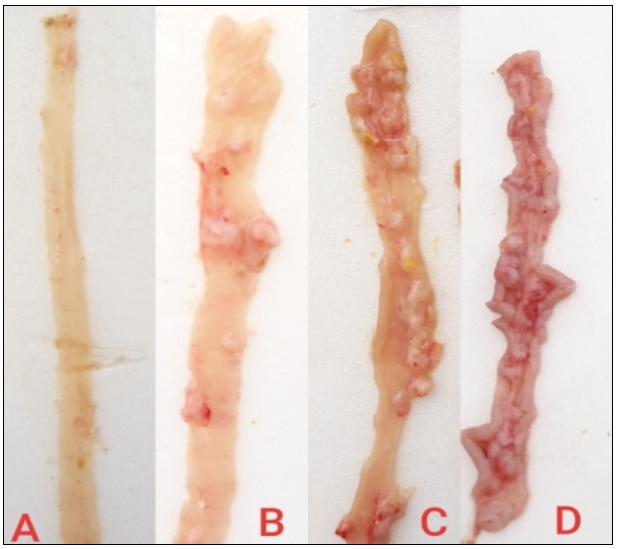


Fig 2: Macroscopic appearance of the distal small intestine: A- Normal control group (score 0) don't show any morphological changes, B- Ind control group (score 7) showing Lesions having diameter more than 2 mm (number 5–10), C- NAC (500 mg/kg) treated group (score 7) showing Lesions having diameter more than 2 mm (number 5–10), NAC (1g/kg) treated group (score 8) showing Lesions having diameter more than 2 mm (number>10)

Table 3: Macroscopic score of different experimental groups

Group Macroscopic Score	Normal control	Indo control	NAC treated (500mg/kg)	NAC treated (1g/kg)
0	5(83.33%)			
1	1(16.67%)			
2				
3				
4				
5				
6		6(85.71%)	4(50%)	3(50%)
7		1(14.29%)	4(50%)	1(16.67%)
8				2(33.33%)

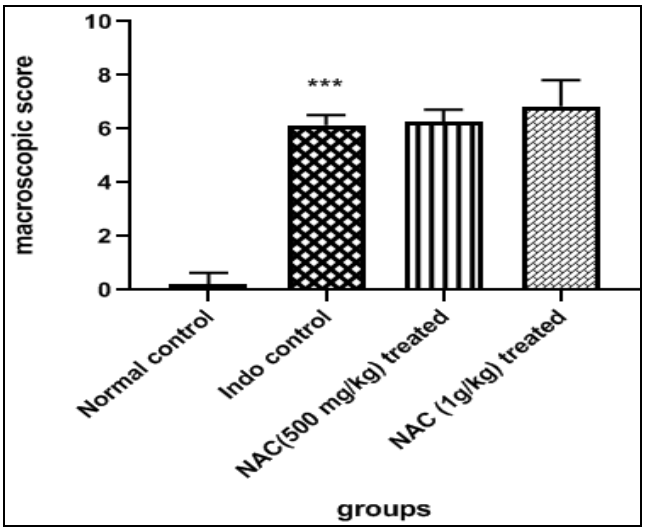


Fig 3: Effect of NAC combined on small intestinal macroscopic score. Data are expressed as mean±S.E.M. *** Significant difference as compared to normal control group at p<0.001, # Significant difference as compared to combination group

Histopathological study

The distal small intestine specimen of 50 % of rats in normal control group revealed an intact architecture, while the distal small intestine specimen of 50 % of rats from this group revealed increased inflammatory cells infiltration, the inflammation was mild to moderate.

On the other hand the distal small intestine specimen of Indo control group revealed increased inflammatory cell infiltration, transmural inflammation, lymphocytic aggregate, cryptitis and ulcerations.

There was statistical significance comparing with normal control group (p=0.0084).

Administration of NAC in both doses revealed increase in the severity of inflammation and injury induced by Indo especially at dose 1 g/kg (Figure4) (Table4) with no statistical significance comparing with Indo control group [NAC (500mg/kg): p= 0.8785, NAC (1g/kg): p=0.6920] (Figure5).

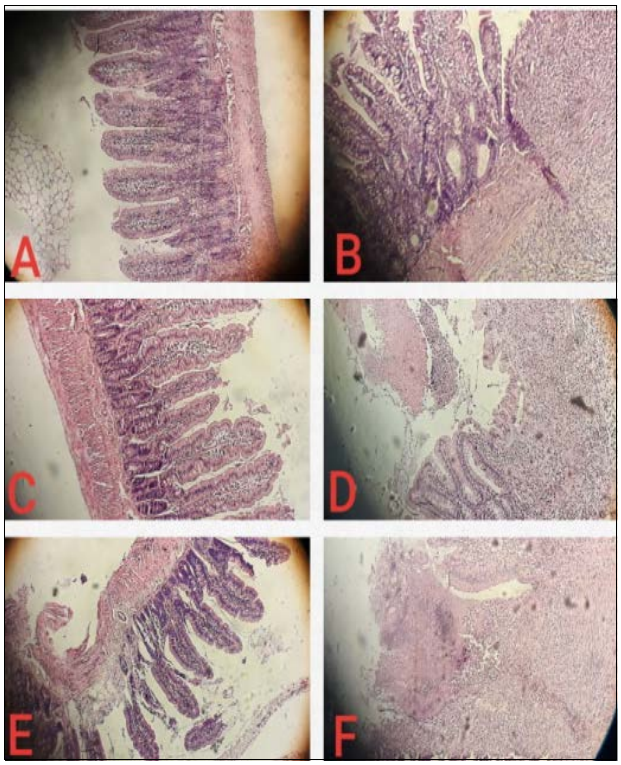


Fig 4: Histological appearance of jejunum tissue sections, original magnification ×10

Normal control group (grade 0) shows an intact architecture, B- Indo control group (grade 5) shows focal cryptitis, transmural inflammation and ulceration, C- NAC (500mg/kg) treated group (grade 2) shows moderate increased inflammatory cells infiltration, D- NAC (500mg/kg) treated group (grade 6) shows transmural inflammation, lymphocytic aggregate, ulceration and necrosis, E- NAC (1g/kg) treated group (grade 3) shows transmural inflammation, F- NAC (1g/kg) treated group(grade 6) shows transmural inflammation, lymphocytic aggregate ulceration and necrosis

Table 4: Microscopic score of different experimental groups

Group Microscopic Score	Normal control	Indo control	NAC treated (500mg/kg)	NAC treated (1g/kg)
0	3 (50%)			
1	1 (16.67%)		2 (25 %)	
2	2 (33.33%)	2 (28.57%)	1 (12.5 %)	1(16.76%)
3		2 (28.57%)		2(33.33%)
4				1(16.76%)
5		3 (42.86%)	4 (50%)	
6			1 (12.5 %)	2(33.33%)
7				
8				
9				
10				
11				

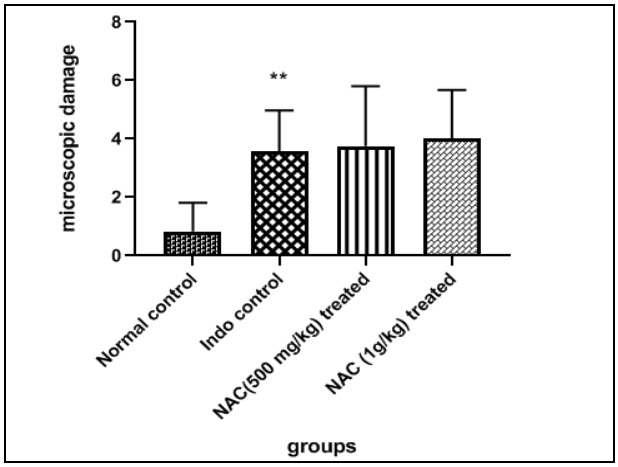


Fig 5: the effects of NAC on small intestinal macroscopic score in Indo induced inflammatory bowel disease model in rats. Data are expressed as mean ± S.E.M. *** Significant difference as compared to normal control group at p<0.001, # Significant difference as compared to combination group

Biochemical assays

Indo induced oxidative stress in the small intestine, which was evaluated by SOD activity and GSH levels.

Indo decreased SOD activity and GSH levels in the distal small intestine, there was statistical significance comparing with normal control group (SOD: p=0.0413, GSH: p=0.0223).

In NAC treated group (500 mg/kg), SOD activity was increased and the levels of GSH were decreased, but there was no statistical significance when the previous findings were compared with Indo control group (SOD: p= 0.1927, GSH: p=0.2138).

In NAC treated group (1g/kg), SOD activity and GSH levels were decreased, but there was no statistical significance when the previous findings were compared with Indo control group (SOD: p= 0.4992, GSH: p= 0.2996) (Table5).

Table 5: The effects of NAC on lipid peroxides, GSH and SOD activity in Indo induced inflammatory bowel disease model in rats

Group Parameter	SOD activity	GSH levels (µM/g of tissue)
Normal control	0.588333±0.058675	1.925±0.213239
Indo control	0.175714±0.05719*	1.391429±0.137207*
NAC treated (500 mg/kg)	0.41625±0.129985	1.12875±0.111731
NAC treated (1g/kg)	0.1025±0.07317	1.156667±0.057135

Values are given as mean± S.E.M. values are statistically significant at *P<0.05 between normal and Indo control groups

Discussion

Indo-induced jejunoileitis in Rats shares similar clinical and histological characteristics with human IBD, yet the precise pathophysiological mechanisms may be different [9]. It has been published that there are similarities between ileal Crohn's disease and Indo-induced experimental jejunal ulcer in the rat [17].

Several factors seem to be important in the pathogenesis of Indo-induced ulceration. Initial epithelial damage is mediated in part by inhibition of protective prostaglandins such as prostaglandin E1, prostaglandin E2, and prostacyclin [18]. Prostaglandins (PG) deficiency, resulting from Indo-induced inhibition of PG synthetase, lowers resistance of intestinal mucosa, allowing penetration of noxious agents such as bacteria, bacterial toxins, and/or bile acids which cause inflammation, necrosis, and ulceration of the intestine [19]. Direct epithelial injury by Indo is probably also a contributing factor. Biliary secretion of Indo is injurious to the intestinal mucosa; the combination of bile and Indo lyses intestinal epithelial cells *in vitro* [18]. The administration of Indo results in generation of free radicals in enterocytes, possibly as a result of mitochondrial dysfunction produced and the infiltration of neutrophils into the mucosa [20].

YAMADA *et al* have shown that one injection of Indo (7.5 mg/kg) produced acute injury and inflammation in the distal jejunum and proximal ileum that were maximal at three days and completely resolved within one week. Two daily subcutaneous injections of Indo produced a more extensive and chronic inflammation that lasted in an active form in more than 75 % of the rats for at least two weeks [21]. This study has shown that administration of two daily subcutaneous injections of Indo (9 mg/kg) was suitable to induce the injury in the small intestine.

In this study Indo-induced inflammation in the small intestinal tissues, as evidenced by body weight loss, reduction in food intake, increases in small intestine weight / length ratio, changes in biochemical parameters which include depletion of GSH and decreased SOD activity. The macroscopic results revealed adhesions, erosion, edema, and hemorrhagic spots. The microscopic score revealed increased inflammatory cells infiltration, transmural inflammation, lymphocytic aggregate, cryptitis and ulcerations.

The present study highlights the effect of NAC in two doses (500mg/kg/day and1g/kg/day) on Indo induced rat model of inflammatory bowel disease.

The effect of NAC on body weight improvement wasn't observed, on the contrary NAC (1g/kg) caused body weight loss greater than Indo control group. In addition NAC in both doses caused increase in small intestine weight / length ratio which was greater at dose 1g/kg indicating increase in the severity of inflammation but there was no statistical significance comparing with Indo control group. Macroscopic and Microscopic score in NAC treated groups (group III and group IV) revealed increase in the severity of the gross lesion especially at dose 1g/kg with no statistical significance comparing with Indo control group.

Regarding of the effect of NAC on antioxidant status, it was observed that SOD activity was increased in NAC (500mg/kg) treated group, but it was decreased in NAC (1g/kg) treated group. The level of GSH was decreased in NAC treated group in both doses. The decrease in GSH levels in NAC treated groups indicates that the oxidative stress in the small intestine tissues was increased.

All these previous findings are in agreement with many previous studies: Akgun *et al* showed that NAC substantially reduced the degree of colonic injury, probably

by regulating free radical production and inhibiting inflammation. It may, therefore, have a role in the treatment of inflammatory bowel disease [22]. Guijarro *et al* showed that combined therapy (NAC and mesalamine) produces a clinical improvement of UC patients [23]. Siddiqui *et al* confirmed that treatment of Trinitrobenzenesulfonic acid (TNBS) induced colitis with NAC plus 5-aminosalicylic acid (5-ASA) was superior to treatment with either agent alone in reducing colonic inflammation and in promoting mucosal repair [24]. Cetinkaya *et al* proved that the NAC administration intraperitoneally or intrarectally to the rats with acetic acid induced colitis can reduce the extent of colonic mucosal injury, attenuate the increase in Myeloperoxidase (MPO) activity and Malondialdhyde (MDA) levels and restore diminished antioxidant enzymes and substance such as SOD, catalase (CAT) and GSH [25]. Wang *et al* showed that Dietary supplementation with NAC can alleviate acetic acid (AA) induced colitis in a porcine model through regulating anti-oxidative responses [26]. Uraz *et al* showed that NAC inhibited not only oxidant damage but also proinflammatory cytokines, and improved the colonic inflammation induced by AA in rats [27]. Amrouche-Mekkioui *et al* showed the role of NAC as a scavenger of phagocytes-derived reactive oxygen species in dextran sodium sulfate (DDS) colitis, suggesting that NAC might be protective in oxidative inflammatory bowel disease and colorectal cancer [28]. Azooz *et al* showed that NAC has anti-inflammatory effects in the acute phase of TNBS-niduced colitis [29]. Ramzan *et al* showed that NAC at a dose of 800 mg/day appears to be efficacious and safe in mild to moderate CD [30]. Hou *et al* proved that NAC supplementation alleviates lipopolysaccharide (LPS)-induced intestinal inflammation [31]. Lee *et al* showed that NAC as a feed additive can enhance livestock intestinal health by modulating intestinal infammation, permeability, and wound healing under LPS-induced dysfunction [32].

In this study NAC in both doses especially at dose 1g/kg increased the inflammation and oxidative stress induced by Indo. This is agreement with Sprong *et al* who proved that high doses of NAC aggravate LPS toxicity, increasing the dose of NAC, protection against LPS toxicity was apparently overruled by pro-oxidant effects, the decrease in GSH at 6 h and 12 h after LPS injection, strongly suggest further oxidation of GSH by oxidative stress induced by high-dose NAC. This effect of NAC is supported by the considerable literature reporting that low molecular-weight thiols are pro-oxidants as well as antioxidants [33].In addition Najaf *et al* has shown that High dose of NAC administration not only did not improve patients' outcome, but also raised the risk of inflammation [34]. Another possible mechanism is mucolytic effect of NAC. Sharpe *et al* has shown that a brief 10 minute exposure of the normal gut to the mucolytic NAC was associated with a significant and approximately two-fold increase in gut permeability. Loss of the mucus layer, especially in the presence of pancreatic proteases, is sufficient to induce gut injury and increase gut permeability [35].

Conclusion

In this study conclude that NAC didn't enhance intestinal inflammation induced by Indo (rat model of IBD) in both doses, in contrast rising in inflammation and oxidative stress was observed in NAC treated groups especially at dose 1g/kg, this action probably due to mucolytic and pro-oxidant effect of NAC at high doses. The study suggests more investigations about this effect of NAC at high doses, and need to study its effect in low doses on this model of IBD.

Acknowledgement

The authors are thankful to Damascus University, faculty of pharmacy for providing funding and the necessary assistance. The authors are also thankful to AL-Assad university hospital for providing the assistance.

Declaration of Interest

The authors report no conflict of interest.

References

1. Blumberg SR. CURRENT Diagnosis & Treatment Gastroenterology, Hepatology, & Endoscopy, Inflammatory Bowel Disease: Immunologic Considerations, 2009, 11-21.
2. Chougule NB, Nitve SA, Koumaravelou K. Phytochemical Investigation and Screening for Inflammatory Bowel Disease Activity of Ethanolic Extract of Kariyat. Pharmacogn J. 2018; 10(3):602–610.
3. Gathungu NG, Cho HJ, Crohn's Disease and Ulcerative Colitis From Epidemiology and Immunobiology to a Rational Diagnostic and Therapeutic Approach, The Genetics of Inflammatory Bowel Disease, 2012, 3-16.
4. Hagar HH, El Medany A, El Eter E, Arafa M. Ameliorative effect of pyrrolidinedithiocarbamate on acetic acid-induced colitis in rats. Eur J Pharmacol. 2007; 554(1):69-77.
5. Tian T, Wang Z, Zhang J. Pathomechanisms of Oxidative Stress in Inflammatory Bowel Disease and Potential Antioxidant Therapies. Oxid Med Cell Longev, 2017, 1-18.
6. Silva MA, Porras M, Jury J, Vergara P, Perdue MH. Characterization of ileal dendritic cell distribution in a rat model of acute and chronic inflammation. Inflamm Bowel Dis. 2006; 12(6):457-470.
7. Nandi J, Saud B, Zinkievich JM, Palma DT, Levine RA. 5-Aminosalicylic acid improves indomethacin-induced enteropathy by inhibiting iNOS transcription in rats. Dig Dis Sci. 2008; 53(1):123-132.
8. Vemu B, Selvasubramanian S, Pandiyan V. Emu oil offers protection in Crohn's disease model in rats. BMC Complement Altern Med [Internet]. 2016; 16(1):1-9. Available from: http://dx.doi.org/10.1186/s12906-016-1035-y
9. Nandi J, Saud B, Zinkievich JM, Yang ZJ, Levine RA. TNF-α modulates iNOS expression in an experimental rat model of indomethacin-induced jejunoileitis. Mol Cell Biochem. 2010; 336(1-2):17-24.
10. Šalamon Š, Kramar B, Marolt TP, Poljšak B. Medical and Dietary Uses of N-Acetylcysteine. Antioxidants. 2019; 8(111):2-16.
11. Kelly GS. Clinical Applications of N-acetylcysteine N-acetylcysteine. Alternative Medicine Review. 1998; (2):114-127.
12. Atalay F, Odabasoglu F, Halici M, Cadirci E, Aydin O, Halici Z, *et al*. N-Acetyl Cysteine Has Both Gastro-Protective and Anti-Inflammatory Effects in Experimental Rat Models: Its Gastro-Protective Effect Is Related to Its *in Vivo* and *in Vitro* Antioxidant Properties. J Cell Biochem. 2016; 117(2):308-319.

13. Jagtap AG, Shirke SS, Phadke AS. Effect of polyherbal formulation on experimental models of inflammatory bowel diseases. J Ethnopharmacol. 2004; 90(2-3):195-204.
14. Nakhai LA, Mohammadirad A, Yasa N, Minaie B, Nikfar S, Ghazanfari G, *et al*. Benefits of Zataria multiflora Boiss in experimental model of mouse inflammatory bowel disease. Evidence-based Complement Altern Med. 2007; 4(1):43-50.
15. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. BBA - Gen Subj. 1979; 582(1):67-78.
16. Li X. Improved pyrogallol autoxidation method: A reliable and cheap superoxide-scavenging assay suitable for all antioxidants. J Agric Food Chem. 2012; 60(25):6418-6424.
17. Anthony A, Pounder RE, Dhillon AP, Wakefield AJ. Similarities between ileal Crohn's disease and indomethacin experimental jejunal ulcers in the rat. Aliment Pharmacol Ther. 2000; 14(2):241-245.
18. Elson CO, Sartor RB, Tennyson GS, Riddell RH. Experimental Model of Inflammatory Bowel Disease. Gastroenterology. 1995; 109: 1344-1367.
19. Derelanko JM, Long FJ. Effect of Corticosteroids on Indomethac in Induced Intestinal Ulceration in the Rat. Digestive Diseases and Sciences. 1980; 25:823-829
20. Basivireddy J, Vasudevan A, Jacob M, Balasubramanian KA. Indomethacin-induced mitochondrial dysfunction and oxidative stress in villus enterocytes. 2002; 64:339-349.
21. Yamada D, Deitch E, Specian DR, Perr AM, Sartor B, Grisham BM *et al*. Mechanisms of acute and chronic intestinal inflammation induced by indomethacin. Inflammation. 1993; 17(6):641-662.
22. Akgun E, Çaliskan C, Celik HA, Ozutemiz AO, Tuncyurek M, Aydin HH *et al*. Effects of N-acetylcysteine treatment on oxidative stress in acetic acid-induced experimental colitis in rats. J Int Med Res. 2005; 33(2):196-206.
23. Guijarro LG, Mate J, Gisbert JP, Perez-Calle JL, Marín-Jimenez I, Arriaza E. *et al*. N-acetyl-L-cysteine combined with mesalamine in the treatment of ulcerative colitis: Randomized, placebo-controlled pilot study. World J Gastroenterol. 2008; 14(18):2851-2857.
24. Siddiqui A, Ancha H, Tedesco D, Lightfoot S, Stewart CA, Harty RF *et al*. Antioxidant therapy with N-acetylcysteine plus mesalamine accelerates mucosal healing in a rodent model of colitis. Dig Dis Sci. 2006; 51(4):698-705.
25. Cetinkaya A, Bulbuloglu E, Kurutas EB, Ciralik H, Kantarceken B, Buyukbese MA *et al*. Beneficial effects of N-acetylcysteine on acetic acid-induced colitis in rats. Tohoku J Exp Med. 2005; 206(2):131-139.
26. Hou Y, Wang L, Yi D, Ding B, Yang Z, Li J, *et al*. N-acetylcysteine reduces inflammation in the small intestine by regulating redox, EGF and TLR4 signaling. Amino Acids. 2013; 45(3):513-522.
27. Uraz S, Tahan G, Aytekin H, Tahan VN.-acetylcysteine expresses powerful anti-inflammatory and antioxidant activities resulting in complete improvement of acetic acid-induced colitis in rats. Scand J Clin Lab Invest. 2013; 73(1):61-66.
28. Amrouche - Mekkioui I, Djerdjouri B. N-acetylcysteine improves redox status, mitochondrial dysfunction, mucin-depleted crypts and epithelial hyperplasia in dextran sulfate sodium-induced oxidative colitis in mice. Eur J Pharmacol [Internet]. 2012; 691(1-3):209-217. Available from: http://dx.doi.org/10.1016/j.ejphar.2012.06.014
29. Azooz GO, Silva De A, Rampton D, Ballinger B Anne. Anti-inflammatory Effect of N-acetylcysteine (NAC) in a Model of Colitis, AGA Abstracts. 2003; 124(4):495.
30. Ramzan NN, Leighton LJ, HeJgh LR, MorJarty C, Clin M *et al*. Efficacy of N-Acetylcysteine in Crohn's Disease - An Open Label Pilot Study, AGA Abstracts. 2001; 120(5):280
31. Hou Y, Wang L, Yi D, Ding B, Yang Z, Li J, *et al*. N-acetylcysteine reduces inflammation in the small intestine by regulating redox, EGF and TLR4 signaling. Amino Acids. 2013; 45(3):513-522.
32. Lee SI, Kang KS. N-acetylcysteine modulates lipopolysaccharide-induced intestinal dysfunction. Sci Rep. 2019; 9(1):1-10.
33. Sprong RC, Winkelhuyzen-Janssen AML, Aarsman CJM, Van Oirschot JFLM, Van Der Bruggen T, Van Asbeck BS. Low-dose N-acetylcysteine protects rats against endotoxin-mediated oxidative stress, but high-dose increases mortality. Am J Respir Crit Care Med. 1998; 157(4 PART I):1283-1293.
34. Najafi A, Mojtahedzadeh M, Ahmadi KH, Abdollahi M, Mousavi M, Chelkeba L, *et al*. The immunological benefit of higher dose N-acetyl cysteine following mechanical ventilation in critically ill patients. DARU, J Pharm Sci. 2014; 22(1):1-9.
35. Sharpe SM, Qin X, Lu Q, Feketeova E, Palange DC, Dong W, *et al*. Loss of the intestinal mucus layer in the normal rat causes gut injury, but not toxic mesenteric lympH. 2011; 34(5):475-481.