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Effect of Amlodipine Combined with N-acetylcysteine on Crohn's Disease Model in Rats

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Abstract: This study highlights the effect of Amlodipine combined with N-acetylcysteine on Crohn's disease model in rats. Model of Crhon's disease was induced by subcutaneous Indomethacin administrated at a dose rate of 9 mg/kg for two days at 24h intervals. N-acetylcysteine (500mg/Kg), Amlodipine (10 mg/kg) and N-acetylcysteine (500 mg/kg) combined with Amlodipine (5mg/kg) were administrated for seven consecutive days beginning 24 h after the first Indomethacin injection. The small intestinal injury was assessed by small intestine weight/length ratio, macroscopic damage, histological study, as well as by biochemical measurement of reduced glutathione, lipid peroxides, and superoxide dismutase activity. The results showed that Administration of Amlodipine combined with N-acetylcysteine decreased small intestine weight/length ratio, macroscopic and microscopic small intestinal damage scores. Superoxide dismutase activity was increased; lipid peroxidation was decreased, but reduced glutathione levels were decreased in the combination group. No statistical significance was observed when previous findings were compared with Indomethacin control group ($p>0.05$). This study also showed that administration Amlodipine combined with N-acetylcysteine significantly decreased macroscopic score comparing with N-acetylcysteine administration alone. Conclude that combination of Amlodipine with N-acetylcysteine didn't produce obvious enhancement in the intestinal injury induced by Indomethacin (rat model of Crhon's disease), but this combination attenuated the injury comparing with N-acetylcysteine alone in this model of Crhon's disease.

Keywords: Crohn's disease - Superoxide dismutase - Reduced glutathione - Lipid peroxides.

1. Introduction

Crohn's disease (CD) is a chronic and relapsing inflammatory disease of unknown etiology (Boulton et al., 2011). It's one of two severe chronic inflammatory bowel disorders. (The other form of inflammatory bowel disease is ulcerative colitis). CD can affect any part of the gastrointestinal tract from the mouth to the anus (Friedman et al., 2010), although distal ileal, ileocaecal, and colonic are the most common distributions (Boulton et al., 2011) with symptoms which may include diarrhea, hematochezia, and abdominal pain (Aberra et al., 2010). Inflammation can extend through all areas of the gut wall, and about 60% of cases include granulomatous inflammation (Boulton et al., 2011). The disease has a relapsing and remitting course with most patients requiring medication and surgery (Chan et al., 2015). The etiology of CD is unknown but the disease is

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believed to manifest from genetic predisposition, immune dysregulation, and the environment triggers (Aberra et al., 2010). Oxidative stress (OS) is considered as one of the etiologic factors involved in several signals and symptoms of inflammatory bowel diseases (IBD) (Moura et al., 2015), direct oxidative damage to intestinal mucosal cells and aggravation of inflammatory stress are two important mechanistic aspects of oxidative stress in IBD. Such interactive mechanisms between oxidative stress and inflammation provide a strong basis for the development of the combination therapies of both antioxidants and anti-inflammatory drugs in the management of IBD (Zhu et al., 2012).

Systemic administration of Indomethacin (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 (Silva et al., 2006). It induced enteritis shares clinical, histological, and pathophysiological characteristics with CD (Goyal et al., 2014). Several authors reported that the histomorphological picture of Indo induced inflammation has a similar picture to that of CD (Jurjus et al., 2004). 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 (Vemu et al., 2016).

Amlodipine is a third generation dihydropyridine-type calcium channel blocker commonly used for the treatment of hypertension (Mohammed et al., 2016). Experimental studies have shown that Amlodipine can inhibit inflammatory cytokines and enhance antioxidant defenses (El Morsy et al., 2015).

N-acetylcysteine (NAC), a precursor of reduced glutathione (GSH), has been in clinical use for more than 30 years, primarily as a mucolytic. NAC is being studied and utilized in conditions characterized by decreased GSH or oxidative stress (Kelly., 1998) .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 (Atalay et al., 2016).

2. Materials and Methods

2.1 Materials

Indomethacin (FarmaSino pharmaceuticals, Jiangsu, China), Amlodipine (PRUDENCE PHARMA CHEM, Ankleshwar, India), N-acetylcysteine (Wuhan Grand Hoyo Company Ltd, China), 5,5'-dithiobis (2- nitrobenzoic acid) (Sigma-Aldrich, Darmstadt, Germany), Tris-hydrochloride (ROTH, Karlsruhe, Germany), Pyrogallol (Merck, Darmstadt, Germany), 2-Thiobarbituric acid (Merck, Darmstadt, Germany), Trichloroacetic acid (Merck, Darmstadt, Germany).

2.2 Animals 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 five 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: Amlodipine treated group (6 rats in this group) received Amlodipine dissolved in physiological saline (10 mg/kg body weight po) for seven consecutive days beginning 24 h after the first Indo injection.

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Group V: combination group (7 rats in this group) received 500 mg/kg of NAC plus 5 mg/kg of Amlodipine for seven consecutive days beginning 24 h after the first Indo injection. Groups three through five 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.

2.3 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

2.4 Macroscopic characters (Table1) (Jagtap et al., 2004)

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 les (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)

2.5 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 aggregate/Follicle, Necrosis, Granuloma, Cryptitis, Crypt abscess and epithelial lesions (erosions, ulcers) (Nakhai et al., 2007).

2.6 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 (TCA) 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 4^{o} C and supernatant was used for assay GSH levels and SOD activity.
The remaining of third portion of tissue homogenate was used for the estimation of Lipid peroxides levels.

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2.6.1 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 (Moron et al., 1997). It is expressed as µM of GSH per gram of tissue.

2.6.2 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 (Li, 2012).

2.6.3 Assay of lipid peroxidation (TBARS)

Lipid peroxidation, an indicator of mucosal injury induced by reactive oxygen species was measured as thiobarbituric acid reactive substance. Briefly, 0.5 ml of small intestinal tissue homogenates prepared were reacted with 2 ml of TBA reagent containing 0.375% TBA, 15% trichloroacetic acid and 0.25 N HCl. Samples were boiled for 15 min, cooled and centrifuged. Absorbance of the supernatants was measured by spectrophotometer measured at 532 nm (33). The concentration of MDA was calculated by the absorbance coefficient of MDA–TBA complex (1.56×10^5 M/cm) and expressed in µmol/100 g of tissue (Hagar et al., 2007) (Kheradmand et al., 2009).

3. 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.

4. Results

4.1 Small intestine weight / length ratio

Small intestine weight / length ratio is indirect reliable marker of the small intestinal inflammation. The increase in this ratio in Indo control group was observed, there was statistical significance comparing with normal control group (p=0.0372). Amlodipine treated group revealed decrease in small intestine weight / length ratio with no statistical significance comparing with Indo control group (p=0.1521). NAC treated group revealed increase in small intestine weight / length ratio with no statistical significance comparing with Indo control group (p=0.7667). Amlodipine combined with NAC treated group revealed decrease in the small intestine weight / length ratio with no statistical significance comparing with Indo (p=0.5178) control group, in addition no statistical significance was observed comparing with NAC treated group (p=0.3369), and Amlodipine treated group (p=0.4084) (Table 2).

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Table 2: Effect of Amlodipine combined with NAC on the small intestine weight/length ratio in crhon's disease model in rats

Parameter Group	small intestine weight / length ratio
Normal control	0.08 ±0.007572
Indo control	0.119± 0.006444*
NAC treated	0.123875±0.015591
Amlodipine treated	0.093333±0.00619
Amlodipine +NAC treated	0.108±0.015065

Values are given as mean± S.E.M. values are statistically significant at *P<0.05 between normal and Indo control groups. Abbreviations: Indo=Indomethacin, NAC=N-acetylcysteine.

4.2 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. Theses 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. Amlodipine treated group reduced the severity of the gross lesion with no statistical significance comparing with Indo control group (p=0.4172). NAC treated group didn't reveal reduction in the severity of the gross lesion, on the contrary they increased it, there wasn't statistical significance comparing with Indo control group (p= 0.7829). Amlodipine combined with NAC treated group revealed reduction in the severity of the gross lesion in some rats (Fig.1) (Table 3). There was no statistical significance comparing with Indo control group (p=0.0524). Statistical significance comparing with NAC treated group was observed (p=0.0215), but there was no statistical significance comparing with Amlodipine treated group (p=0.2927) (Fig. 2).

Table 3. Macroscopic score of different experimental groups

Group Macroscopic Score	Normal control	Indo control	NAC treated	Amlodipine treated	Amlodipine + NAC treated
0	5(83.33%)				1(14.29%)
1	1(16.67%)			1 (16.67%)	1(14.29%)
2					
3					2(28.57%)
4					
5					
6		(85.71 %)	4(50%)	5(83.33 %)	3(42.86%)
7		1(14.29 %)	4(50%)		
8					

Abbreviations: Indo=Indomethacin, NAC=N-acetylcysteine

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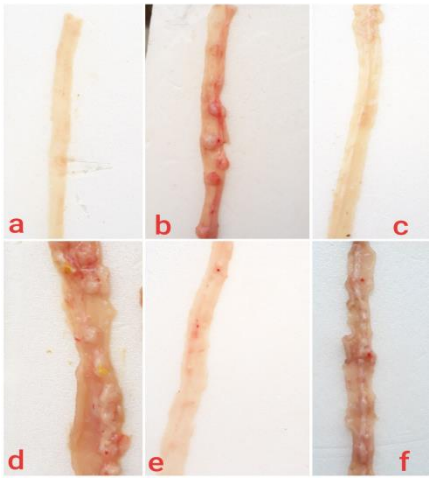

Figure 1: Macroscopic appearance of the distal small intestine: a- Normal control group (score 0), b- Indomethacin control group (score 7), c- Amlodipine treated group (score 1), d- N-acetylcysteine treated group (score 7), e- Amlodipine+N-acetylcysteine treated group (score 1), f- Amlodipine + N-acetylcysteine treated group (score 6)

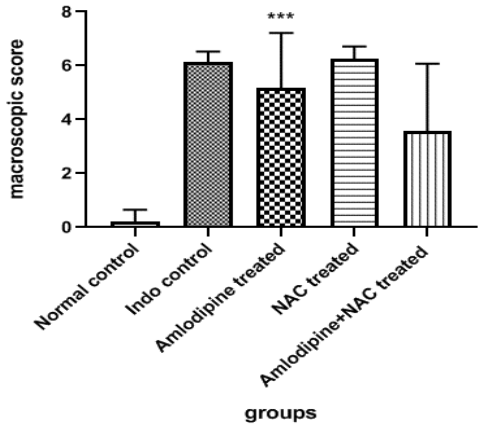

Figure 2: Effect of Amlodipine combined with N-acetylcysteine on small intestinal macroscopic score. Data are expressed as mean±S.E.M. *** Significant difference as compared to normal control group at $p<0.001$, # Significant difference as compared to combination group.

4.3 Histopathological Study

There was a good correlation between macroscopic and histological scores in each study group. 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

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ulcerations. There was statistical significance comparing with normal control group (p=0.0084). Administration of Amlodipine as therapy revealed in some rats reduce in the severity of the injury of the distal small intestine induced by Indo with no statistical significance comparing with Indo control group (p=0.1499). Administration of NAC revealed increase in microscopic injury induced by Indo with no statistical significance comparing with Indo control group (p=0.7669). Administration of Amlodipine combined with NAC revealed decrease in the microscopic injury induced by Indo (Fig. 3) (Table 4), but there was no statistical significance comparing with Indo control group (p=0.4859), in addition no statistical significance was observed comparing with NAC treated group (p=0.4181), and Amlodipine treated group (p=0.4473) (Fig. 4).

Table 4: Microscopic score of different experimental groups.

Group Macroscopic Score	Normal control	Indo control	NAC treated	Amlodipine treated	Amlodipine +NAC treated
0	3 (50 %)			2 (33.33 %)	1 (14.29%)
1	1 (16.67 %)		2 (25 %)		
2	2 (33.33 %)	2 (28.57 %)	1 (12.5 %)	1 (16.67 %)	2 (28.57%)
3		2 (28.57 %)		2 (33.33 %)	1 (14.29%)
4				1 (16.67 %)	2 (28.57%)
5		3 (42.86 %)	4 (50%)		1 (14.29%)
6			1 (12.5 %)		
7					
8					
9					
10					
11					

Abbreviations: Indo=Indomethacin, NAC=N-acetylcysteine

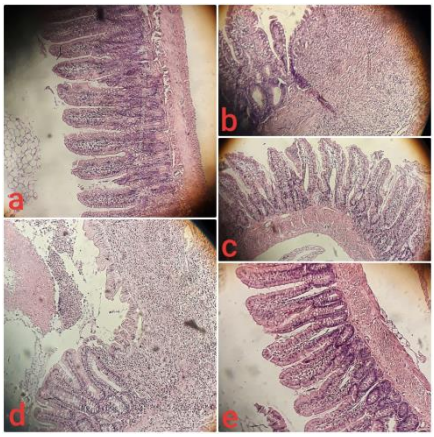

Figure 3: Histological appearance of jejunum tissue sections, original magnification ×10
a - Normal control group (grade 0) shows an intact architecture, b- Indomethacin control group (grade 5) shows focal cryptitis, transmural inflammation and ulceration, c- Amlodipine treated group (grade 4) shows transmural inflammation and lymphoid aggregate in muscular layer, , d- N-acetylcysteine (500mg/kg) treated group (grade 6) shows transmural inflammation, lymphoid aggregate ulceration and necrosis, e- Amlodipine + N-acetylcysteine treated group (grade 2) shows mild transmural inflammation

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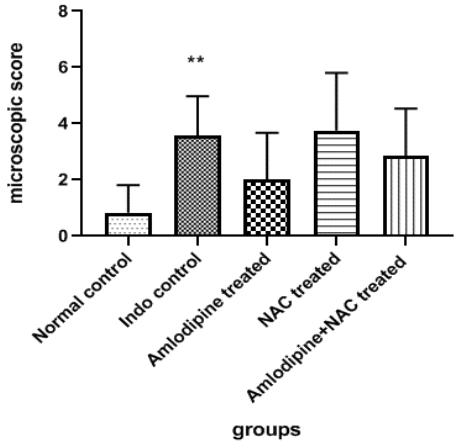

Figure 4: Effect of Amlodipine combined with N-acetylcysteine on small intestinal microscopic score. Data are expressed as mean±S.E.M. **Significant difference as compared to normal control group at p<0.01

4.4 Biochemical Assays

Indo induced oxidative stress in the small intestine, which was evaluated by lipid peroxidation, SOD activity and GSH levels. Indo increased the level of lipid peroxides, 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, Lipid peroxides: p=0.0294). In NAC treated group, SOD activity was increased, the levels of GSH and lipid peroxides were decreased, but there was no statistical significance when the previous findings were compared Indo control group (SOD: p= 0.1927, GSH: p=0.2138, Lipid peroxides: p= 0.7467). Amlodipine treated group revealed decrease in the level of lipid peroxides, increase in SOD activity, its effect on GSH levels in the distal small intestine wasn't observed. There was no statistical significance comparing with Indo control group (SOD: p=0.6746, GSH: p=0.5996, Lipid peroxides: p=0.6308). Amlodipine combined with NAC treated group revealed decrease in the levels of lipid peroxides, increase in SOD activity, whereas the levels of GSH weren't affected. There was no statistical significance when the previous findings were compared with Indo control group (SOD: p=0.6746, GSH: p=0.5996, Lipid peroxides: p=0.6308), in addition no statistical significance was observed comparing with NAC treated group (SOD: p=0.1927, GSH: p=0.3453, Lipid peroxides: p=0.0890), and Amlodipine treated group (SOD: p=0.3477, GSH: p=0.8107, Lipid peroxides: p=0.2412) (Table 5).

Table 5: Effect of Amlodipine combined with NAC on lipid peroxides, GSH and SOD activity in Indo induced Crohn's disease model in rats.

Parameter Group	**SOD activity**	**GSH levels (µM/g of tissue)**	**Lipid peroxides (µmol/100 g of tissue)**
Normal control	0.588333±0.058675	1.925±0.213239	44.33333±4.333333
Indo control	0.175714±0.05719*	1.391429±0.137207*	79.71429±16.53238*

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Amlodipine treated	0.3193330.190946	1.133333±0.162714	72.16667±12.53107
NAC treated	0.41625±0.129985	1.12875±0.111731	75±5.682052
Amlodipine + NAC treated	0.525429±0.202145	1.327143±0.226545	51.85714±9.818419

Values are given as mean± S.E.M. values are statistically significant at $^{*}P<0.05$ between normal and Indo control groups. Abbreviations: Indo=Indomethacin, NAC=N-acetylcysteine, GSH=Glutathione, SOD=Superoxide dismutase.

5. Discussion

Since the exact etiology of IBD is not known yet, a number of animal models have been developed over past decades in order to study the possible mechanism involved in pathogenesis of disease and new therapeutic targets (Goyal et al., 2014). In general, an appropriate or an optimal animal model should display certain key characteristics: the gut should exhibit morphological alterations, inflammation, symptoms and signs, pathophysiology, and course similar or identical to the human IBD (Jurjus et al., 2004). Nonsteroidal anti-inflammatory drug (NSAIDs) have also been associated with relapse of classic inflammatory bowel disease, and NSAID administration to humans and animals produces an enteropathy similar functionally and pathologically to that observed in chronic inflammatory bowel disease. Because of these similarities, NSAID-induced intestinal inflammation has been used as an experimental model of inflammatory bowel disease (Battarbee et al., 1996). Due to the ease of induction and the wide relevance of the model to clinical recurrence, Indo was used in the present study, which was reported to cause clinical condition that is similar in picture as well as mechanism to that of CD (Vemu et al., 2016). It has been suggested that Indo-induced intestinal injury is a consequence of cyclooxygenase inhibition and a subsequent mucosal prostaglandin deficiency (Battarbee et al., 1996), 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 (DERELANKO et al., 1980) . 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 (ELSON et al., 1995). 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 (Basivireddy et al., 2002).

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

The present study highlights the effect of Amlodipine combined with NAC on CD model in rats.

NAC alone didn't ameliorate the injury induced by Indo in small intestine (model of CD), in contrast it was observed increase in the gross lesion that was evidenced by macroscopic and microscopic score with no statistical significance comparing with Indo control group. This isn't in harmony with several studies: AKGUN et al who showed that NAC substantially reduced the degree of colonic injury, probably by regulating free radical production and inhibiting inflammation (Akgun et al., 2005). Guijarro et al who showed that combined therapy (NAC and mesalamine) produces a clinical improvement of UC patients (Guijarro et al., 2008). Cetinkaya et al proved that the NAC administration to the rats with acetic acid induced colitis can reduce the extent of colonic mucosal injury (Cetinkaya et al., 2005). Azooz et al showed that NAC has anti-inflammatory effects in the acute phase of Trinitrobenzene sulfonic acid (TNBS)-induced colitis. This treatment may be worth

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further assessment in the treatment of human IBD (Azooz et al., 2003). Ramzan et al showed that NAC at a dose of 800 mg/day appears to be efficacious and safe in mild to moderate CD (Ramzan et al., 2001). 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 (Lee et al., 2019).

The decrease in GSH level indicates that there was increase in the oxidative stress in the small intestine tissue. This is in harmony with SPRONG et al who proved that high doses of NAC aggravate lipopolysaccharide (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 (Sprong et al., 1998). One of the possible mechanisms that NAC didn't enhance the injury and increased the inflammation 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 (Sharpe et al., 2011).

Amlodipine administration alone didn't produce obvious enhancement in this model of CD. Although there was decrease in small intestine weight / length ratio, macroscopic and microscopic score, increase in SOD activity, decrease in lipid peroxides levels in the small intestine tissue in Amlodipine treated group, but no statistical significance was observed when previous findings were compared with Indo control group. This is in agreement with several authors who reported the effect of Amlodipine on IBD model in rats. El Morsy et al who showed that Amlodipine has attenuating effect in ulcerative colitis in rats (El Morsy et al., 2015). Rajinikanth et al proved that Amlodipine possess significant reduction in inflammation against acetic acid induced ulcerative colitis in mice (Rajinikanth et al., 2015). Also this isn't in harmony with several authors who have also reported the anti-oxidant effect of Amlodipine. Mahajan et al who proved that Amlodipine improved the status of oxidative stress as shown by a decrease in MDA and increase in SOD levels in essentials hypertension patients (Mahajan et al., 2007). El Morsy et al proved that Amlodipine exerts anti oxidative effects in vitro and in vivo by inhibiting the oxidizability of the cell membrane and low-density lipoproteins. This effect was mediated by quenching free radicals due to its highly lipophilic properties and its chemical structure (El Morsy et al., 2015).

Administration of Amlodipine combined with NAC attenuated the small intestinal inflammation which was evidenced by decrease small intestine weight / length ratio, macroscopic and microscopic score; this attenuating was greater than group treated with NAC alone. This combination also attenuated the biochemical changes by increased SOD activity and decreased the levels of lipid peroxides; this was greater than administration of NAC or Amlodipine alone. The levels of GSH weren't affected by combination of Amlodipine with NAC. There was no statistical significance when compared previous findings with Indo control group, Amlodipine and NAC treated groups (except there was statistical significance when compared macroscopic score of combination group with NAC treated group).

6. Conclusion

Taken together, the results of this study showed that combination of Amlodipine with NAC didn't produce obvious enhancement in the intestinal injury induced by Indo (rat model of CD) but this combination attenuated the injury comparing with NAC alone in this model of CD in rats. The study suggests more investigations on the effect of Amlodipine combined with NAC in this model of CD, and administration of NAC in low doses.

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