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MODULATORY ROLE OF N-ACETYL-CYSTEINE ON GASTRIC MUCOSAL LESION AND HAEMATO-BIOCHEMICAL CHANGES IN ALBINO WISTAR RATS SUBJECTED TO INDOMETHACIN TREATMENT

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ABSTRACT

Gastric ulcer is caused by multifaceted etiological factors such as environmental and indiscriminate use of non-steroidal anti-inflammatory drugs (NSAIDs) such as Indomethacin. N-acetyl-cysteine (NAC) is an antioxidant that protects the lipid bio-membrane against oxidative stress. This study investigated the effect of NAC on gastric mucosal lesion and haemato-biochemical changes in albino Wistar rats subjected to indomethacin treatment. Twenty (20) adult male rats, were divided into five (5) groups; Group I (Control): Were administered with distilled water/kg/Bdw, Group II: Indomethacin 40 mg/kg in 0.5 % carboxymethylcellulose (Ulcer group), Group III: Received 2.5 ml/kg of 0.5% CMC, Group IV: Received NAC 500 mg/kg/Bdw orally + Indomethacin (500 mg/kg), Group V: Received Ranitidine 50 mg/kg/Bdw + Indomethacin (40 mg/kg). All treatment lasted for 7 days. Three hours after last treatment, rats were humanely sacrificed. The stomach and blood samples were collected for physical and biochemical analysis. Data was analysed using ANOVA and $p < 0.05$ was considered significant. The P index of NAC in indomethacin induced ulcer is found to be 75 %. A significant increase ($p < 0.05$) in final body weight was observed in Indomethacin group, when compared to the control, CMC and Ranitidine + Indomethacin groups. A significant ($p < 0.05$) increase in inducible nitric oxide synthase (INOS) concentration was observed in all treatment group, when compared to the control. In conclusion, we surmise that acute administration of Indomethacin increased body weight of rats, which was decreased by CMC and Ranitidine treatments, while NAC treatment failed to improve haemato-biochemical changes in adult Wister rats.

Keywords: Gastric Ulcer, N-Acetyl-Cysteine, Oxidative Stress, Indomethacin

INTRODUCTION

Gastric ulcer is an illness that affects a considerable number of people worldwide due to stress and misuse of Nonsteroidal anti-inflammatory drugs. Nonsteroidal anti-inflammatory drugs (NSAIDs) are agents clinically used to reduce fever, pain and inflammation. As such, a lot of people indiscriminately used NSAIDS to minimize pain leading to gastrointestinal toxicity (Ribeiro-Rama *et al*., 2009). Furthermore, in disease conditions like rheumatoid arthritis and osteoarthritis, there is high chance of NSAIDS misuse and toxicity. The mechanisms via which NSAIDS induce gastric ulcer involves the inhibition of cyclooxygenase enzymes (COX) resulting in inhibition of gastric prostaglandins synthesis, specifically prostaglandin E_2 (PGE_2) and prostaglandin I_2 (PGI_2) which are the main inhibitors of gastric acid secretion, leading to excessive gastric acid secretion that results in excoriation of the gastric mucosa consequently leading to ulceration of the GI

(Ribeiro-Rama *et al*., 2009; Atalay *et al*., 2016). Therefore, gastric lesions are revealed as an important adverse effect caused by inhibiting prostaglandin biosynthesis (Koc *et al*., 2008). Among NSAIDs, Indomethacin has been widely used to reduce inflammation, pain and fever in humans. It also causes injury on the gastric mucosa due to the inhibition of COX enzymes and suppression of prostaglandins (Muscara *et al*., 2000).

N-acetyl-cysteine (NAC) is a derivative of thiol-containing amino acid that is a precursor for the intracellular antioxidant glutathione. It has potent antioxidant effects and detoxifies reactive neutrophils and enhances the eradication of free radicals either through conjugation or reduction reactions (Jacob, 2015). As stated earlier, Gastric ulcer affect people of all ages, hence, there is need to develop therapeutic and prophylactic strategies that are cheap, readily available with minimal side effects against the disease. N-Acetyl Cysteine is currently a supplement but its gastro protective effects have not been clearly extensively explored although its' antioxidant and anti-inflammatory properties have been established in other disease conditions (Ausama, 2015). Therefore, the purpose of this study is to investigate the modulatory role of NAC on indomethacin induced gastric mucosal injury in Wistar rats pre-treated with NAC as well as its effects on some haematological and biochemical parameters including activities of antioxidant enzymes in gastric tissue. This may reveal new therapeutic strategies for maintaining gastric mucosal integrity thus suggesting new approach that may be validated and adopted in improving outcomes in patients with gastric mucosal injury and inflammation.

MATERIALS AND METHODS

Animals and Management

A total of twenty (20) male rats, 8-12 weeks of age, and weighing 130-180 g were used for this study. The animals were purchased from the Animal House Facility of the Department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Gombe State University Gombe, Nigeria. They were given free access to standard commercial grower's mash feed and water. The mice were allowed to acclimatise to the environment for two weeks before the commencement of the experiment. All experimental protocols were carried out in accordance with the Gombe State University Research policy, ethics and regulations, governing the care and use of experimental animals (NIH Publication no. 85-23, revised 1996). The experiments were conducted in a quiet laboratory from 9: 00 h to 16: 00 h, with light-dark cycle of about 12:12 h.

Experimental Design and Animal Treatment Protocol

Thirty-six (36) hours before the induction of gastric ulcer, animals were deprived of food to allow for complete gastric emptying, but they were allowed access to water *ad libitum*. During fasting, rats were housed each in a separate cage with a wide-raised wall, mesh bottom to prevent coprophagy (Gou *et al*., 2012). The animals have free access to water except the last hour before the experiments. All experiments were performed during the same time of the day to avoid variations due to diurnal rhythms of putative regulators of gastric functions. The animals were randomly classified into eight groups (4 rats per each) as follows:

i. Group I (Control): Animals received distilled water/kg body weight orally for 7 days.
ii. Group II: Animals received Indomethacin in a dose of 20 mg⁄ kg in 2.5ml/kg of 0.5% of carboxymethylcellulose after 7 days of oral administration of distilled water (Ulcer group).

iii. Group III: Animals received 2.5 ml/kg of 0.5 % Carboxymethylcellulose after 7 days of oral administration of distilled water.
iv. Group IV: Animals received N-Acetyl Cysteine (500 mg/kg) body weight orally for 7 days + Indomethacin (40 mg/kg) on the seventh day
v. Group V: Animals received Ranitidine (50 mg/kg) body weight body weight orally for 7 days + Indomethacin (40 mg/kg) on the seventh day

Assessment of Gastric Mucosal Lesions

Three hours after administration of indomethacin, rats of all groups were anaesthetized using (Diazepam 25 mg/kg + Ketamine 75 mg/kg) and humanely sacrificed. The stomach of each animal was rapidly removed, opened by an incision along the greater curvature. The stomachs were rinsed with saline. Gastric tissues were pinned out flat on a cork board. The severity of mucosal lesions was grossly inspected and photograph. Gastric tissues were fixed in 10% formalin, dehydrated and embed in paraffin wax. Paraffin section of 5 µm were cut and stained with haematoxylin and eosin. Histological changes were examined under a light microscope. Ulcer index was determined as follows: Lesion size in millimetres was determined by measuring each lesion at its greatest diameter with a transparent millimetre scale rule (Sadau *et al*., 2015). Five petechiae lesions were considered equal to 1mm lesion. The total length in each group of rats were averaged and expressed as lesion index (Wong *et al*., 2002). Preventive index (%) = ulcer index of control- ulcer index of treated / ulcer index of control x 100.

Biochemical Analysis of Gastric Mucosa

Frozen gastric mucosal tissues were rinsed with ice cold isotonic saline. The tissue was then ground in a cold glass mortar and homogenized with ice cold 100 mM phosphate buffer (pH 7.4; 1g of tissue/5ml), containing 1mM EDTA and (10µg⁄ml) indomethacin. The lysate was then centrifuged at 2000g for 10 minutes at 2 ^{O}C to 8 ^{o}C. The supernatant was then transferred to a new tube, and used for the following biochemical analyses.

Quantification of Rat mucosal Malondialdehyde (MDA) concentration

The rat's MDA Elisa assay kit (Fn: ER1878) was used to assess the concentration of MDA in rat gastric mucosa, based on the principle of competitive-ELISA detection method. The standard, test samples and control wells were set on the pre-coated plate respectively, and their positions recorded. Exactly 50 µL of the standard, blank or sample were added per well. The blank well was added with sample/standard dilution buffer. Immediately, 50 µL Biotin-labelled antibody working solution was added to each well. The plate was covered with a seal and shaken gently to mix and incubated for 45 minutes at 37^{o} C. Thereafter, the plate was removed and drained off liquid. Each well was filled with washing buffer solution and allowed to stand for 1 minutes, after which it was blot off. The washing was repeated three times. 100 ul of HRP-Streptavidin Conjugate (SABC) working solution was added to each well. The wells were sealed and incubated for 30 minutes at 37^{o} C, after which the cover was removed and the plate was washed 5 times with a wash buffer. 90 µL of TMB substrate was added to into each well, and incubated in the dark for 10-20 minutes for colour development. The plate was removed after 20 minutes and 50 µL stop solution was added to each well to stop the reaction. Colour changes were observed from blue to yellow. The absorbance of each well was measured one by one at 450 nm, 10 minutes after adding stop solution. The optical density was measured

and concentration of the samples was determined using MyAssays software.

Quantification of Rat mucosal Super Oxide Dismutase (SOD) concentration

The rat's SOD Elisa assay kit (Fn: ER0332) was used to assess the concentration of SOD in rat gastric mucosa, based on the principle of competitive-ELISA detection method according to the manufacturer's protocol as described above.

Quantification of Rat mucosal Inducible Nitric Oxide Synthase (INOS) concentration

The rat's INOS Elisa assay kit (Fn: ER0150) was used to assess the concentration of INOS in rat gastric mucosa, based on the principle of competitive-ELISA detection method according to the manufacturer's protocol as described above.

Quantification of Rat mucosal Prostaglandins E2 (PGE2) concentration

The rat's PGE2 Elisa assay kit (Fn: ER0150) was used to assess the concentration of PGE2 in rat gastric mucosa, based on the principle of competitive-ELISA detection method according to the manufacturer's protocol as described above.

Collection of Blood Samples

Blood samples were collected through cardiac puncture (Ebunlomo *et al.,* 2012) and kept in EDTA bottle used for haematological parameters; total blood count, differential blood count and erythrocyte osmotic fragility test.

Determination of Haematological Parameters

Total (Red blood cell count, White blood cell count, haemoglobin count, packed cell volume, platelet counts, mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular concentration) and differential blood counts (monocytes, lymphocytes, eosinophil, basophils, granulocyte) were determined using Biobase (Bk6000) haematology analyser.

Evaluation of Erythrocyte Osmotic Fragility

The erythrocyte osmotic fragility test was determined using the method Faulkner and King, 1970 as described by Asala *et al*. (2011). Briefly, a set of 10 test tubes containing 0.1% to 0.9% concentration of sodium chloride (NaCl) were used. Each tube contained 5ml of the corresponding sodium chloride and was arranged serially in a rack of 10 tubes. 1ml pipette was used to transfer 0.02ml into each of the 10 test tubes in a set. The contents of the test tubes were gently mixed by inverting the test tubes five times and allowing them to stand at room temperature for 30 minutes. Thereafter, the contents of the test tubes were centrifuged at 2000 x g for 10 minutes. The supernatant was transferred in to a glass cuvette and the absorbance of the supernatant was measured at a wavelength 540nm using a spectrophotometer. The percentage haemolysis was calculated according to Faulkner and King (1970) as follows:

$$\text{Percentage haemolysis} = \frac{\text{Optical density of the test}}{\text{Optical density of the standard (Distilled water)}} \times 100$$

Erythrocyte osmotic fragility curve was obtained by plotting percent haemolysis against the saline concentration.

Statistical Analyses

Results obtained were presented Mean $\pm$ S.E.M. Statistical comparison between variables was carried out using analysis of variance (ANOVA). Tukey's post-hoc test

was used to compare the differences between the means values. Values of $p < 0.05$ was considered significant.

RESULTS

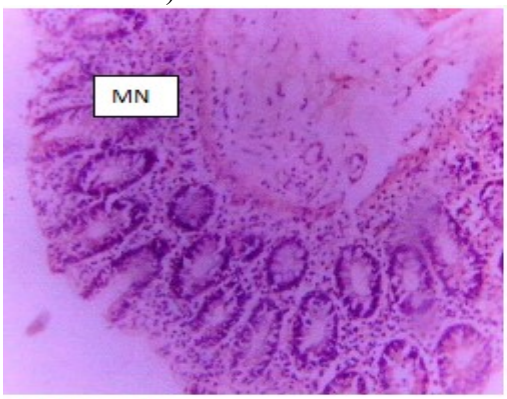
Table 1: Shows Ulcer index and Preventive index of N- acetyl cysteine

GROUP	TREATMENT	ULCER INDEX (mm)	PREVENTIVE INDEX (%)
1	Distilled water	-	
2	Indomethacin alone, positive control	4	
3	CMC, passive control	-	
4	N-acetyl cysteine+ indomethacin	1	75
5	Ranitidine + Indomethacin	1	

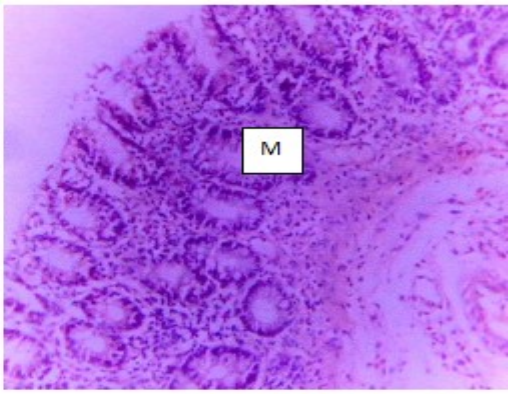
Preventive index of N-acetyl cysteine in indomethacin induced ulcer = (4-1/4) × 100 = 75 %

Results of Histology

GP 1 (Distilled Water)

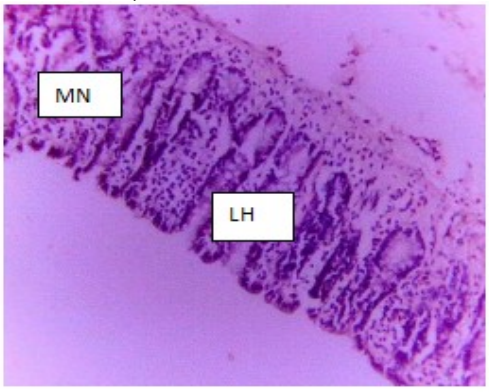


SHOWS SLIGHT MN

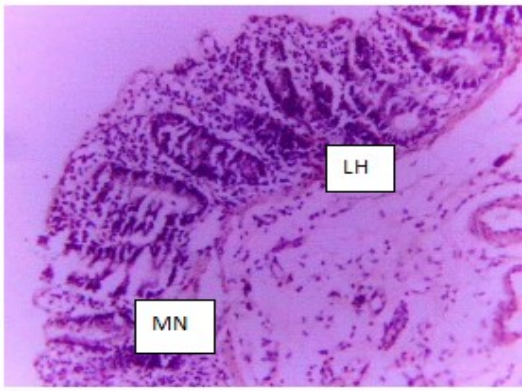


SHOWS NORMAL "M"

GP2 (Indomethacin)

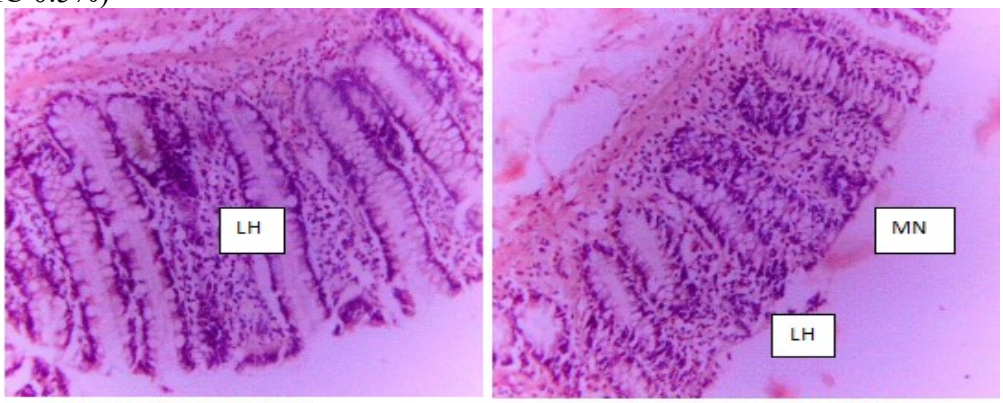


SHOWS SLIGHT MN AND LH
LH



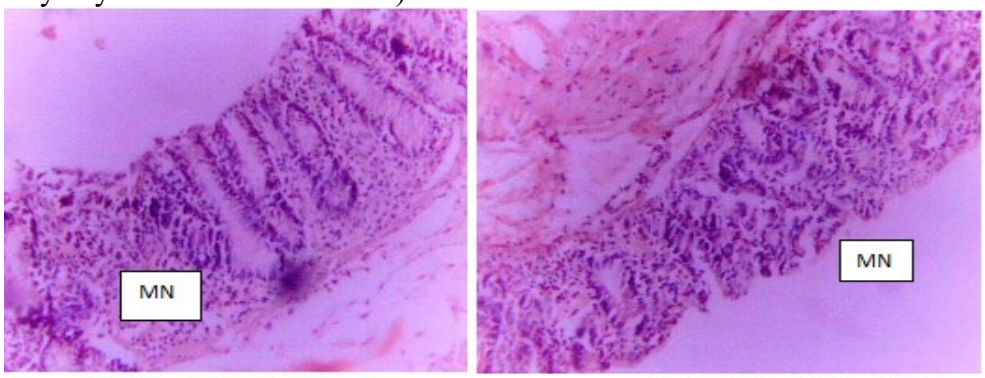
SHOWS SLIGHT MN AND

GP3 (CMC 0.5%)



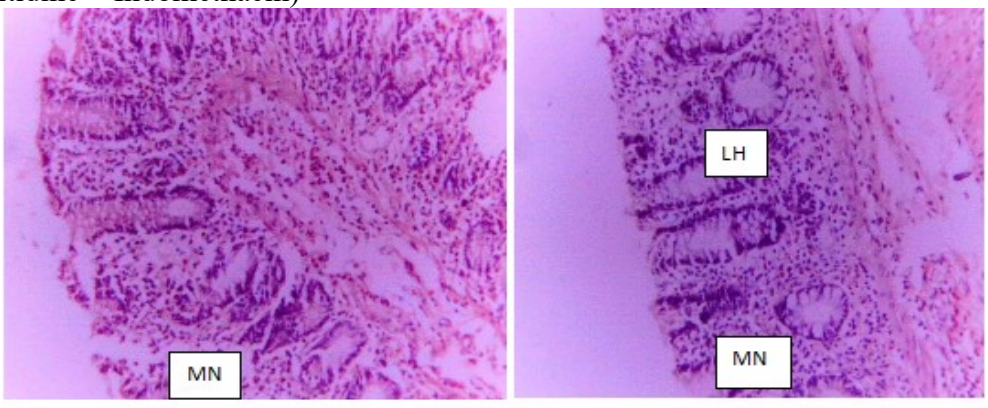
SHOWS SLIGHT LH | SHOWS SLIGHT LH AND MN

GP4 (N-Acetyl Cysteine + Indomethacin)



SHOWS MODERATE MN | SHOWS MODERATE MN

GP5 (Ranitidine + Indomethacin)



SHOWS MODERATE MN | SHOWS SLIGHT MN AND LH

Figure 1: Histological examination of gastric mucosal tissue group 1-5

Keys:
MN: Mucosa Necrosis
LH: Hyperplasia of Inflammatory Cells
LD: Lipofuchsin Deposits

Effect of N-Acetyl-Cysteine on Oxidative Stress Biomarkers in Albino Wistar Rats Subjected to Indomethacin Treatment

Malondialdehyde Concentration

The effect of N-Acetyl-Cysteine on the MDA concentration in rats Subjected to Indomethacin Treatment (Figure 2). There was no significant ($p > 0.05$) change observed in the MDA concentration in all the treatment groups when compared to the control [F (7, 23) = 2.21; $p = 0.07$].

Superoxide Dismutase (SOD) Concentration

The effect of N-Acetyl-Cysteine on SOD concentration in rats Subjected to Indomethacin Treatment (Figure 3). There was no significant ($p > 0.05$) change observed in the SOD concentration in all the treatment groups when compared to the control [F (7, 23) = 0.77; $p = 0.61$].

Inducible Nitric Oxide synthase (INOS) Concentration

The effect of N-Acetyl-Cysteine on INOS concentration in rats Subjected to Indomethacin Treatment (Figure 4). There was significant ($p < 0.05$) increase observed in the INOS concentration in all the treatment groups; Indomethacin (18.17 ± 1.25), CMS (18.76 ± 0.78), NAC + Indomethacin (19.94 ± 0.57), Ranitidine + Indomethacin (19.13 ± 0.23), when compared to the control, (14.23 ± 0.83), [F (7, 23) = 5.33; $p = 0.001$].

Effect of N-Acetyl-Cysteine on Prostaglandins E2 (PGE_2) in Albino Wistar Rats Subjected to Indomethacin Treatment

Prostaglandins E2

The effect of N-Acetyl-Cysteine on the PGE_2 concentration in rats Subjected to indomethacin treatment (Figure 5). There was no significant ($p > 0.05$) change observed in the PGE_2 concentration in all the treatment groups when compared to the control, although there was an increase in the indomethacin group but it was not statistically significant, [F (7, 23) = 2.6; $p = 0.06$].

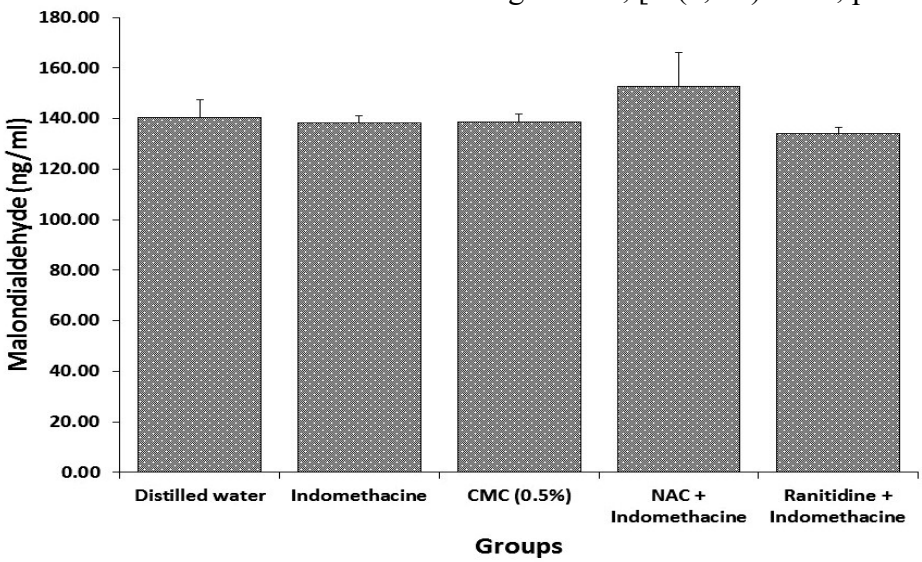


Figure 2: Effect of N-Acetyl-Cysteine on Malondialdehyde Concentration in Albino Wistar Rats Subjected to Indomethacin Treatment.
CMC = Carboxymethyl Cellulose, NAC = N-Acetyl Cysteine, n = 4

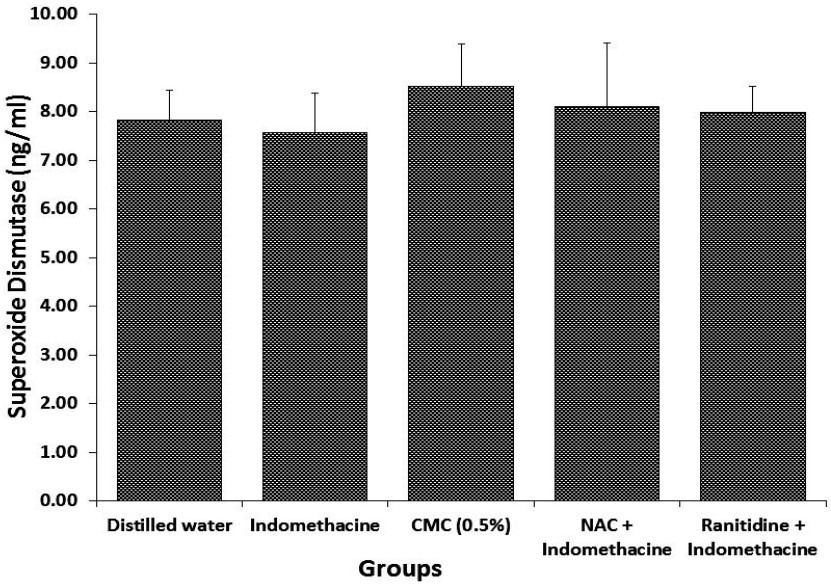


Figure 3: Effect of N-Acetyl-Cysteine on Superoxide Dismutase Concentration in Albino Wistar Rats Subjected to Indomethacin Treatment

CMC = Carboxymethyl Cellulose, NAC = N-Acetyl Cysteine, n = 4

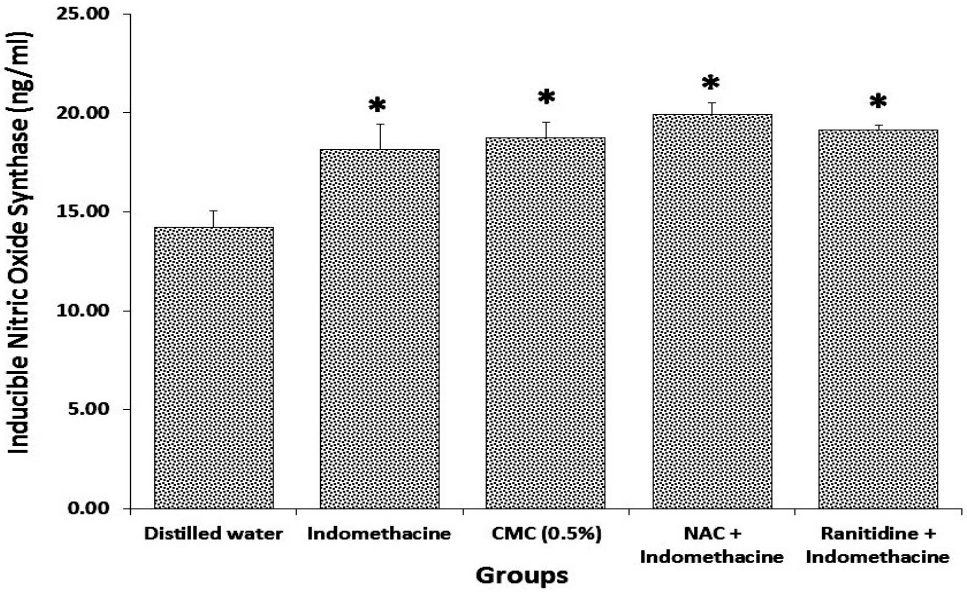


Figure 4: Effect of N-Acetyl-Cysteine on Inducible Nitric Oxide Synthase in Albino Wistar Rats Subjected to Indomethacin Treatment

***** Indicate significant ($p < 0.05$) difference when compared to the control, CMC = Carboxymethyl Cellulose, NAC = N-Acetyl Cysteine, n = 4

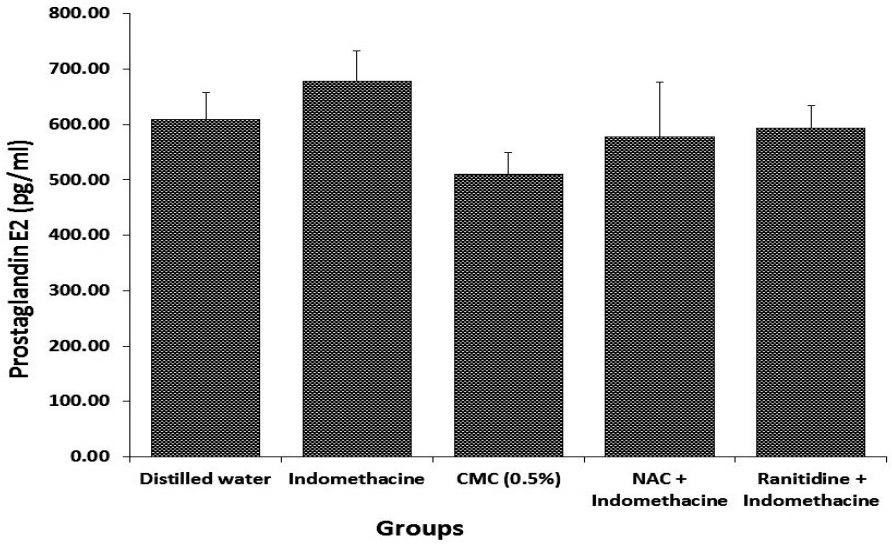


Figure 5: Effect of N-Acetyl-Cysteine on Prostaglandin E_2 Concentration in Albino Wistar Rats Subjected to Indomethacin Treatment

CMC = Carboxymethyl Cellulose, NAC = N-Acetyl Cysteine, n = 4

Effect of N-Acetyl-Cysteine on Erythrocyte Osmotic Fragility Test (EOFT) in Albino Wistar Rats Subjected to Indomethacin Treatment

The effect of N-Acetyl-Cysteine on EOFT in Albino Wistar Rats Subjected to Indomethacin Treatment (Table 2) There was no significant ($p > 0.05$) change observed in EOFT in all the treatment groups when compared to the control, [$F(7, 20) = 1.13$; $p = 0.38$], [$F(7, 20) = 1.58$; $p = 0.19$], [$F(7, 20) = 0.49$; $p = 0.82$], [$F(7, 20) = 0.83$; $p = 0.57$], respectively.

Effect of N-Acetyl-Cysteine on Total Blood Count (TBC) in Albino Wistar Rats Subjected to Indomethacin Treatment

The effect of N-Acetyl-Cysteine on (TBC) in Albino Wistar Rats Subjected to Indomethacin Treatment (Table 3) There was no significant ($p > 0.05$) change observed in TBC in all the treatment groups when compared to the control, [$F(7, 22) = 1.18$; $p = 0.53$], [$F(7, 22) = 0.83$; $p = 0.58$], [$F(7, 22) = 0.18$; $p = 0.98$], [$F(7, 22) = 0.57$; $p = 0.77$], [$F(7, 22) = 0.36$; $p = 0.92$], [$F(7, 22) = 1.65$; $p = 0.17$], [$F(7, 22) = 0.76$; $p = 0.62$], [$F(7, 22) = 0.43$; $p = 0.87$], respectively.

Effect of N-Acetyl-Cysteine on Differential White Blood Count (DWBC) in Albino Wistar Rats Subjected to Indomethacin Treatment

The effect of N-Acetyl-Cysteine on DWBC in Albino Wistar Rats Subjected to Indomethacin Treatment (Table 4) There was no significant ($p > 0.05$) change observed in TBC in all the treatment groups when compared to the control, [$F(7, 22) = 1.18$; $p = 0.35$], [$F(7, 21) = 0.90$; $p = 0.51$], [$F(7, 22) = 0.68$; $p = 0.68$], [$F(7, 22) = 1.13$; $p = 0.37$], [$F(7, 22) = 1.40$; $p = 0.25$], [$F(7, 22) = 0.86$; $p = 0.55$], respectively.

Table 2: Effect of N-Acetyl-Cysteine on Erythrocyte Osmotic Fragility Test in Albino Wistar Rats Subjected to Indomethacin Treatment

GROUPS	0.9 % NaCl	0.7% NaCl	0.5% NaCl	0.4% NaCl	0.3% NaCl	0.2% NaCl
Distilled water	0.00 ± 0.00	31.50 ± 0.95	48.50 ± 0.86	75.25 ± 7.18	91.25 ± 4.69	100.00 ± 0.00
Indomethacin	0.00 ± 0.00	32.50 ± 3.66	53.00 ± 2.94	79.25 ± 3.68	95.50 ± 4.50	100.00 ± 0.00
CMC (0.5%)	0.00 ± 0.00	31.00 ± 2.27	51.50 ± 4.92	75.50 ± 7.59	93.50 ± 3.94	100.00 ± 0.00
NAC + Indomethacin	0.00 ± 0.00	27.33 ± 3.17	47.67 ± 1.33	74.67 ± 2.96	89.33 ± 1.66	100.00 ± 0.00
Ranitidine + Indomethacin	0.00 ± 0.00	31.67 ± 2.90	48.33 ± 1.76	78.00 ± 6.65	91.00 ± 5.85	100.00 ± 0.00

CMC = Carboxymethyl Cellulose, NAC = N-Acetyl Cysteine, NaCl + Sodium Chloride, n = 4

Table 3: Effect of N-Acetyl-Cysteine on Total Blood Count in Albino Wistar Rats Subjected to Indomethacin Treatment

GROUPS	HB g/dl	PCV %	WBC X 10^3/uL	RBC X 10^6/uL	PCT %	MCV f/L	MCH pg	MCHC g/dl
Distilled water	13.20 ± 0.87	39.25 ± 1.84	5.05 ± 0.40	5.78 ± 0.26	0.17 ± 0.02	86.08 ± 2.23	29.68 ± 0.57	34.18 ± 1.93
Indomethacin	12.42 ± 0.58	37.25 ± 1.60	4.93 ± 0.21	6.03 ± 0.10	0.25 ± 0.02	86.68 ± 2.68	28.35 ± 0.95	33.43 ± 0.28
CMC (0.5%)	13.50 ± 0.41	41.5 ± 2.02	4.75 ± 0.46	6.05 ± 0.09	0.18 ± 0.05	86.98 ± 0.49	32.35 ± 2.22	34.13 ± 1.29
NAC + Indomethacin	11.40 ± 0.36	35.67 ± 0.88	4.70 ± 0.20	5.70 ± 0.30	0.27 ± 0.06	84.83 ± 3.26	32.87 ± 2.88	33.40 ± 0.10
Ranitidine + Indomethacin	12.85 ± 0.67	39.50 ± 2.50	4.90 ± 0.14	5.85 ± 0.13	0.18 ± 0.04	87.98 ± 0.34	30.87 ± 0.98	33.05 ± 0.76

CMC = Carboxymethyl Cellulose, NAC = N-Acetyl Cysteine, HB = Haemoglobin, PCV = Packed Cell Volume, WBC = White Blood Cell, RBC = Red Blood cell, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration, n = 4

Table 4: Effect of N-Acetyl-Cysteine on Differential White Blood Count in Albino Wistar Rats Subjected to Indomethacin Treatment

GROUPS	NEUTROPHILS COUNT	NEUTROPHILS (%)	LYMPHOCYTE COUNT	LYMPHOCYTE (%)	MID COUNT	MID (%)
Distilled water	3.08 ± 0.05	34.05 ± 2.88	5.83 ± 0.53	62.88 ± 2.34	0.27 ± 0.06	3.75 ± 0.92
Indomethacin	2.50 ± 0.23	37.20 ± 2.99	5.33 ± 0.54	56.90 ± 2.46	0.45 ± 0.09	5.68 ± 0.51
CMC (0.5%)	2.67 ± 0.14	33.45 ± 1.55	5.63 ± 0.39	61.60 ± 1.56	0.98 ± 0.37	5.08 ± 0.52
NAC + Indomethacin	2.57 ± 0.35	31.90 ± 3.71	5.27 ± 0.38	61.30 ± 4.70	1.07 ± 0.67	5.83 ± 1.35
Ranitidine + Indomethacin	2.83 ± 0.38	33.53 ± 2.87	6.00 ± 0.42	61.53 ± 2.99	0.35 ± 0.03	4.40 ± 0.22

CMC = Carboxymethyl Cellulose, NAC = N-Acetyl Cysteine, MID = Monocytes, Eosinophils and Basophils, % = Percentage n = 4

DISCUSSIONS

This study investigated the modulatory role of N-acetyl-cysteine on gastric mucosal lesion and some haemato-biochemical changes in albino Wistar rats subjected to indomethacin treatment. The preventive index of N-acetyl cysteine on

indomethacin induced ulcer was found to be 75% (Table 1), demonstrating protection of N-acetyl cysteine in gastric mucosal lesion induced by indomethacin in the current study. As reported by Karbasi *et al*. (2013) NAC not only has antioxidant function but also has several other mechanisms of action, including inhibition of neutrophil activation, vasodilation, and reduced microbial attachment According to Reza *et al*. (2014) it also Modulates inflammatory pathways. Results of histological studies revealed slight mucosal necrosis and hyperplasia of inflammatory cells in the indomethacin group while only moderate necrosis was observed in the N-acetyl cysteine + indomethacin treated group, demonstrating the beneficial role of the pre-treatment with N-acetyl cysteine in protection of gastric mucosal lesion in the current study. The control group shows normal mucosal lining with slight necrosis. Indomethacin induced oxidative stress as indicated by increase gastric MDA levels and decreased gastric SOD activity compared to the control group. Pre-treatment with NAC decreased the generation of MDA in gastric tissue, but increased gastric SOD activity. The effect of N-Acetyl-Cysteine on the MDA (Figure 2) and SOD (Figure 3) concentrations in rats subjected to Indomethacin treatment revealed that there was no significant change in the concentrations of both MDA and SOD across the different treatment groups in the current study when compared to the control; this contradicted the finding of Hegab *et al*. (2018), who obtained significant changes. However, a significant ($p < 0.05$) increase in INOS concentration was observed in all the treatment groups, when compared to the control. The present study was in agreement with finding of Kemidi *et al*. (2013) who reported that increases in NO synthase (iNOS) activity is involved in the gastrointestinal mucosal defence. However it is in disagreement with the findings of Hegab *et al*. (2018), who reported decreased in INOS concentration and up regulation of SOD by N-acetyl cysteine in Indomethacin induced gastric mucosa injury in rats. These changes could be transient it is not sufficient enough to cause significant change in MDA concentration which is the final biochemical marker of oxidative stress that results in the lysis of the membranous lipid bilayer. The effect of N-Acetyl-Cysteine on the PGE_2 concentration in rats Subjected to indomethacin treatment presented in figure 5 revealed that there was no significant ($p > 0.05$) change observed in the PGE_2 concentration in all the treatment groups when compared to the control. This is also in disagreement with the findings of Atalay et al. (2016) and Soliman et al. (2017, who reported the anti-inflammatory role of NAC on Indomethacin induced gastric mucosa damage in Wistar rats. However, PGE_2 concentration was observed in the various treatment groups and the control when compared to the indomethacin group, with a trend towards significance. Extending the study, a little further could have resulted to a significance difference. The result of the effect of N-Acetyl-Cysteine on the haematological parameters in rats Subjected to indomethacin treatment presented in table 2, 3, and 4 revealed that there was no significant change observed in erythrocyte osmotic fragility, total and different blood counts respectively, across all the treatment groups when compared to the control. This demonstrated that both indomethacin and N-acetyl cysteine have no significant influence on haematological parameter in the current study. Overall, the results obtained from the current study could be due to the dose of indomethacin used. While we used 20 mg/kg of indomethacin, some investigators up to 50 mg/kg (Jacob et al., 2015; Atalay *et al*., 2016, Soliman *et al*., 2017). This suggests that increase our dose or

elongating the study a little longer could result to significant statistical difference.

CONCLUSIONS

In conclusion, based on the findings from our study, we surmise that acute administration of N-acetyl cysteine failed to improve haemato-biochemical changes in adult Wister rats subjected to indomethacin treatment.

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