DOI: https://dx.doi.org/10.4314/jaaps.v11i2.3

J. Afr. Ass. Physiol. Sci. 11(2): 25-33, December 2023

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences

https://www.ajol.info/index.php/jaaps

Research Article

Modulatory role of N-acetyl-cysteine on gastric mucosal lesions and some biochemical changes in Wistar rats subjected to cold restraint stress

Ibrahim Lai[1], Yakubu Sadau[2] and Mustapha S. Muhammad[1]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, Gombe State University

[2]Department of Human Physiology, Faculty of Basic Medical Sciences, Federal University Dutse

Keywords:

Gastric Ulcer, N-Acetyl-Cysteine, Oxidative Stress, Cold Restrain Stress

* Address for Correspondence:
Email: ysadau@fud.ng.edu

Received: 13 August 2023
Revised: 18 October 2023
Accepted:22 December 2023

ABSTRACT

Background: Gastric ulcer affects many people worldwide and it is caused by many factors such as stress, medications, particularly non-steroidal anti-inflammatory drugs, infections caused by Helicobacter pylori and Cytomegalovirus. Exposure to Cold restraint stress (CRS) has been established to cause oxidative stress leading to cellular death. N-acetyl-cysteine (NAC) is an antioxidant that protects the lipid bio-membrane against oxidative stress. This study investigated the effect NAC on gastric mucosal lesion and some biochemical changes in Wistar rats subjected to CRS. **Methodology:** Sixteen (16) adult male rats were divided into four (4) groups; Group I (Control): Distilled water/Kg Group II: Distilled water + CRS 3½ hrs (Ulcer group), Group III: NAC 500 mg/kg orally + CRS 3½ hrs Group IV: Ranitidine 50 mg/kg + CRS 3½ hrs. All treatment lasted for 7 days while exposure to CRS was for 3½ hours on 7th day. Three hours after exposure of rats to CRS, rats of all groups were euthanized under diazepam and ketamine anesthesia. The stomach and blood samples were collected for physical and biochemical analysis. Data were analysed using ANOVA and $p < 0.05$ was considered significant. **Results:** The P index of NAC in CRS induced ulcer was found to be 66.7 %. A significant increase ($P = 0.001$) in body weight was observed in CRS + Ranitidine group, when compared to the control. A significant ($P = 0.001$) increase was observed in the INOS concentration in NAC + CRS, Ranitidine + CRS, when compared to the control. **Conclusion**: We surmise that acute administration of NAC significantly increased body weight of rats subjected to CRS. The high preventive index of N-acetyl cysteine on CRS induced ulcer was as the result of the antioxidant properties of NAC which might have contributed to its' gastro protection against gastric mucosal lesions.

1. Introduction

Stress is an adaptive physiological response to disruption of homeostasis (Guo *et a*l., 2012). An imbalance between free radical generation and various antioxidant defense systems leading to the accumulation of free radicals is called oxidative stress (Elewa *et al*., 2012). The exposure to cold restraint stress (CRS) situations can stimulate numerous pathways, leading to increased production of oxygen free radicals that generate a cascade of reactions producing lipid peroxidation. Thus, lipid peroxidation is considered as a serious consequence of free radical toxicity leading to profound changes in the membrane structure and function that may even cause cell death (Eqbal *et al*., 2012). One of the reasons for the stress-induced enhancement of free radicals may be the elevation of nitric oxide (NO) production. NO may interact with oxygen, superoxide anion, and thiol compounds, generating reactive nitrogen species (RNS), the Lipid peroxidative effect of NO may be mediated through ONOO- which is a potent and long lived oxidant (Akpinar *et al*., 2008). Nitric oxide (NO) is the main regulator of gastric blood flow that participates in the maintenance of mucosal integrity (Mohamed and Walaah, 2013). It was proposed that the ulcerogenesis possibly depend upon the interplay between ROS generation and NO action in stress (Ishii *et al*., 2000). It has been reported that increases in NO synthase (NOS) activity are involved in the gastrointestinal mucosal defence and also in the pathogenesis of mucosal damage (Kemidi *et al*., 2013).

Cold restraint stress causes gastric mucosal ulceration in rats; the pathological basis for the development of this lesion has been postulated to be multifactorial such as increased gastric acid secretion, inhibition of gastric mucosal prostaglandin synthesis, disruption of gastric mucosal barrier, reduction of gastric mucosal blood flow, inhibition of gastric mucus and bicarbonate secretion (Güzel *et al*., 1998). NSAIDs ulcer occurs mainly due to their local inhibitory effect on gastric prostaglandin E_2 (PGE_2) and prostaglandin I_2 (PGI_2) that are the main inhibitors of gastric acid secretion (Ribeiro-Rama *et al*., 2009). 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, NAC detoxifies reactive neutrophils and enhances the eradication of free radicals through either conjugation or reduction reactions (Ausama, 2015).

The purpose of this study was to investigate if NAC can prevent gastric mucosal injury in Wistar rats' pre-treated before the induction of the ulcer through CRS as well as its effects on some biochemical parameters including activities of antioxidant enzymes in both gastric tissue and liver. This may reveal new therapeutic strategies for maintaining gastric mucosal integrity, thus improving outcomes in patients with gastric mucosal injury and inflammation.

2. Materials and Methods

2.1. Drugs and chemicals

N-acetyl-cysteine (100g) of analytical grade was purchased from Sigma chemical Co. St. Louis, MO, USA (A7250). Diazepam and Ketamine were purchased from Kumbi Pharmacy, Gombe, Nigeria, carboxymethyl cellulose (CMC) (Product No: 27929, BDH Laboratory Chemicals Limited, Poole, England), 70% alcohol, PGE2 (ER1800), SOD (ER0332), MDA (ER1878) and INOS (ER0150) Fine Test ELISA biochemical assay kits were used in the study.

2.2. Animals and management

A total of sixteen (16) male rats, 8-12 weeks of age, and weighing 130-180 g were used for this study. The rats 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 rat chow and water. The rats 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). Rats were housed each in separate cage with a wide-raised, mesh bottom to prevent coprophagy and were exposed to 12 hours each of natural daylight and darkness

2.3. Experimental Design and Animal Treatment Protocol

Thirty-six (36) hours before the onset of gastric ulcer induction, 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 (Shu *et al*., 2012). The animals had 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 follow:

i. Group I (Control): Animals received distilled 5 ml/kg body weight orally for 7 days.
ii. Group II: Animals were given distilled water 5 ml/kg body weight orally for 7 days and exposed to Cold Restrain Stress (CRS) for 3½ hours on the seventh day (Ulcer group)
iii. Group III: Animals received N-Acetyl Cysteine (500 mg/kg) body weight orally for 7 days and exposed to Cold Restrain Stress (CRS) for 3½ hours on the seventh day
iv. Group IV: Received Ranitidine (50 mg/kg) body weight body weight orally for 7 days + exposed to Cold Restrain Stress (CRS) for 3½ hours on the seventh day

CRS: cold restrained stress to induce gastric ulcer, the rats were immobilized in individual restraint boxes and subjected to cold (4±1C^{o}) stress for 3.5hours (Dalia, M. A. and Mai, M. H. 2011).

2.4. Assessment of gastric mucosal lesions

Three hours after exposure of rats to CRS, rats of all groups were anaesthetized using (Diazepam 25 mg/kg + Ketamine 75 mg/kg) and humanely euthanized. The stomach of each animal was rapidly removed, opened with 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 embedded in paraffin wax. Paraffin sections 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 lengths 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.

2.5. Biochemical analysis of gastric mucosa

Frozen gastric mucosal tissues were rinsed with ice cold isotonic saline. The tissues were 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 2000 x g for 10 minutes at 2 ^{o}C to 8 ^{o}C. The supernatant was then be transferred to a new tube, and used for the following biochemical analyses.

2.5.1. 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 in the pre-coated plate respectively, and their positions recorded. Exactly 50 µL of the standard, blank or sample were added per well. The blank wall was added to 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 of liquid. Each well was filled with washing buffer solution and allowed to stand for 1 minute, after which it was blotted off. The washing was repeated three times. Thereafter 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. Thereafter 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 under 450 nm, 10 minutes after adding stop solution. The optical density was measured and concentration of the samples was determined using MyAssays software.

2.5.2. 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.

2.5.3. 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.

2.5.4. 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.

2.6. Statistical Analyses

The results obtained were presented as Mean $\pm$ Standard Error of Mean (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 mean values. A value of $P < 0.05$ was considered significant.

3. Results

Effect of N-Acetyl-Cysteine on Gastric Mucosal Lesion and Some Haemato-Biochemical Changes in Wistar rats subjected to cold restrain stress

3.1. Ulcer Index

Table 1 below shows the ulcer indices and ulcer inhibition capacity of N- acetyl cysteine treated rats. N- acetyl cysteine inhibited ulcer by 66.7%.

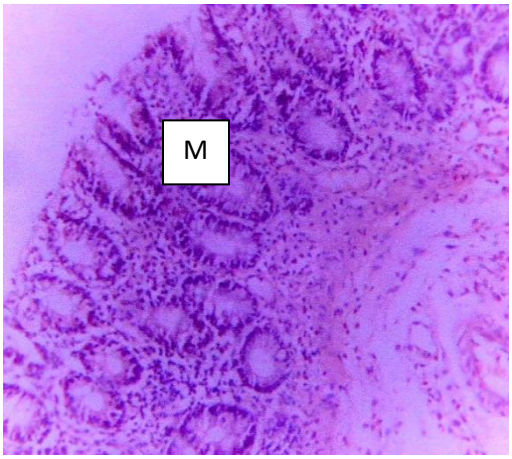
Table 1. Ulcer index and Preventive index of N- acetyl cysteine

GROUP	TREATMENT	ULCER INDEX IN (mm)	PREVENTIVE INDEX (%)
I	Control	0	
II	Cold Restraint Stress	3	
III	N-Acetyl Cysteine + Cold Restraint Stress lesion	1	66.7 %
IV	Ranitidine + Cold Restrain Stress	0	

3.2. Results of Histology

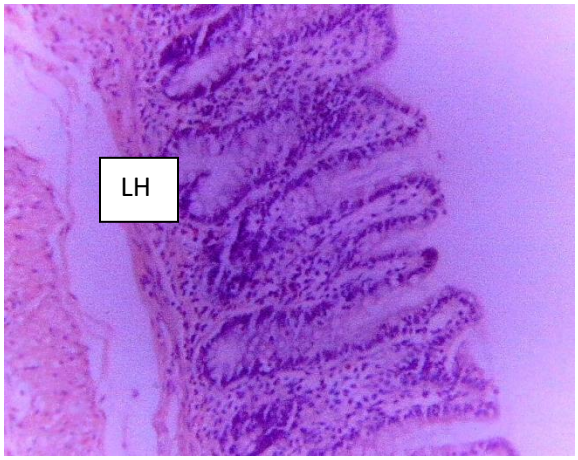
Histopathological findings of the gastric mucosa obtained in this study, GP1 (Distilled Water) showed an intact cellular architecture while GP2 (CRS) active control showed slight hyperplasia of inflammatory cells (LH). GP3 (NAC + CRS) Shows slight mucosa necrosis (MN) and LH this showed the protection against these histophatological changes, induced by CRS in rats. GP4 (Ranitidine + CRS) Showed moderate hyperplasia of inflammatory cells and lipofuchsin deposits (LH and LD).

GP 1 (Distilled Water)



SHOWS NORMAL MUCOSA (M)

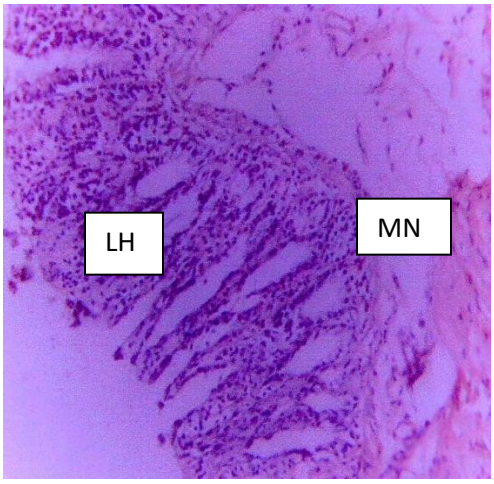
GP2 (CRS



SHOWS SLIGHT LH

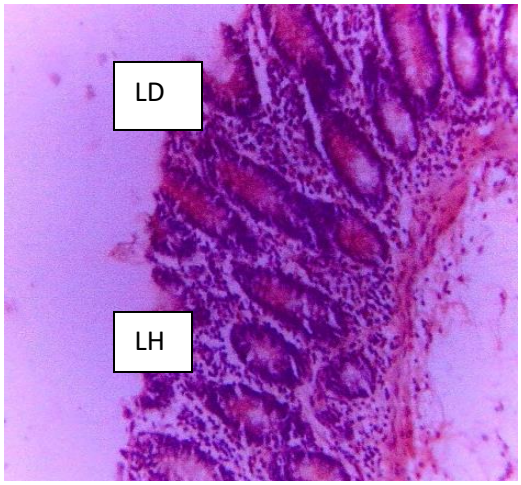
(H&E Mag x100)

GP3 (NAC + CRS)



SHOWS SLIGH MN AND LH

GP4 (Ranitidine + CRS)



MODERATE LH AND LD

(H&E Mag x100

MEANING OF CODES

MN is mucosa necrosis; LH is hyperplasia of inflammatory cells; LD is lipofuchsin deposits

3.3. Effect of N-Acetyl-Cysteine on the changes in body weight of Wistar rats subjected to cold restraint stress (CRS)

Body Weight Changes

The effect of N-Acetyl-Cysteine on the changes in body weight of Wistar rats subjected to CRS (Figure 1) A significant ($P < 0.05$) increase in base-line body weight (body weight 1) was observed in the CRS group (181.00 ± 2.08g) treatment group when compared to the Control, (128.50 ± 14.50g), [F (7, 20) = 2.65; p = 0.04]. A significant increase in the final body weight (body weight 2) was also observed in the CRS + Ranitidine group (217.25 ± 6.18g) when compared to the control (154.50 ± 8.89g). However, a decrease in final body weight (body weight 2) was also observed in the CRS (178.33 ± 2.85g) and NAC + CRS groups (151.33 ± 3.33g) when compared to the Ranitidine + CRS group (217.25 ± 6.18g), [F (7, 20) = 11.8; p = 0.001].

3.4. Effect of N-Acetyl-Cysteine on Oxidative Stress Biomarkers in Wistar rats subjected to cold restrain stress (CRS)

Malondialdehyde Concentration

The effect of N-Acetyl-Cysteine on the MDA concentration in rats subjected to CRS (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) Activity

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

3.5. Inducible Nitric Oxide synthase (INOS) Concentration

The effect of N-Acetyl-Cysteine on INOS concentration in rats subjected to CRS (Figure 4). There was a significant ($p < 0.05$) increase observed in the INOS concentration in NAC + CRS (18.00 ± 0.42), Ranitidine + CRS (19.00 ± 0.40), when compared to the control, (14.23 ± 0.83), [F (7, 23) = 5.33; p = 0.001].

3.6. Effect of N-Acetyl-Cysteine on Prostaglandins E2 (PGE_2) in Albino Wistar Rats Subjected to Cold Restrain Stress (CRS) Prostaglandins E2 Concentration

The effect of N-Acetyl-Cysteine on the PGE_2 concentration in rats Subjected to CRS (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 [F (7, 23) = 2.6; p = 0.06].

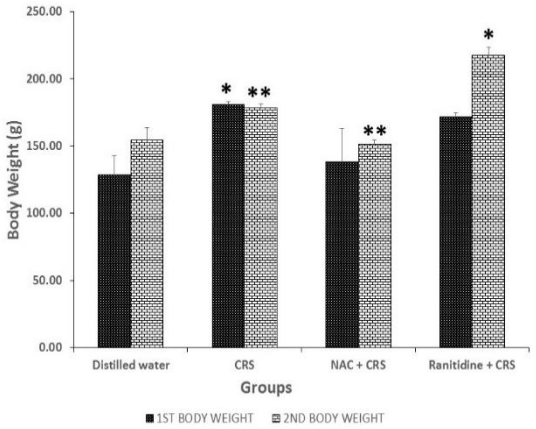


Figure 2: Effect of CRS on body weight in Wistar rats subjected to cold restraint stress.
NAC = N-Acetyl Cysteine, n = 4
** = are significantly ($P < 0.001$)
* = are significantly ($P < 0.05$)

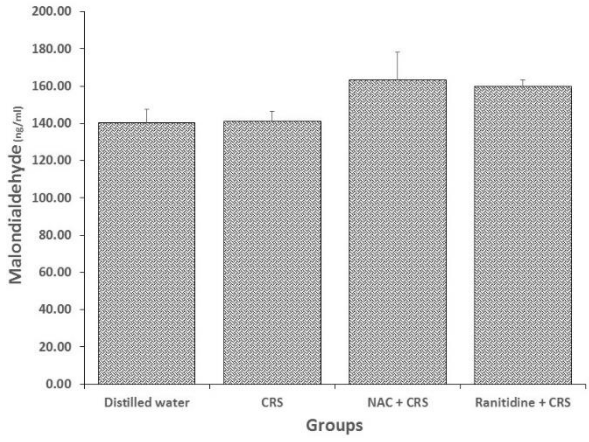


Figure 3: Effect of N-Acetyl-Cysteine on malondialdehyde Concentration in Wistar rats subjected to cold restraint stress
NAC = N-Acetyl Cysteine, n = 4

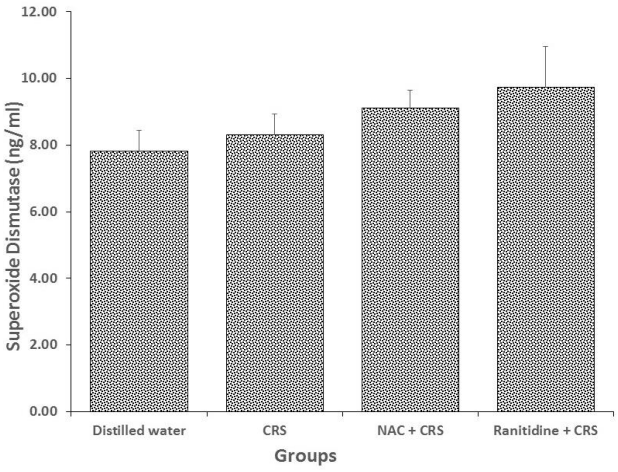


Figure 4: Effect of N-Acetyl-Cysteine on Superoxide Dismutase activity in Wistar Rats subjected to cold restraint stress. NAC = N-Acetyl Cysteine, n = 4

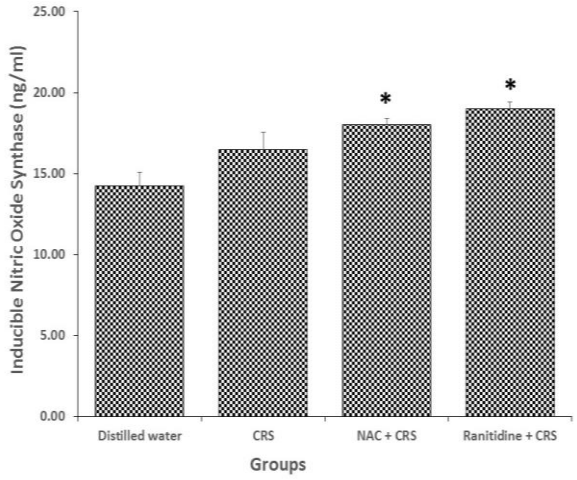


Figure 5: Effect of N-Acetyl-Cysteine on Inducible Nitric Oxide Synthase in Wistar rats subjected to cold restraint stress.
***** Indicate significant ($p < 0.05$) difference
NAC = N-Acetyl Cysteine, n = 4
* = are significantly ($P < 0.05$)

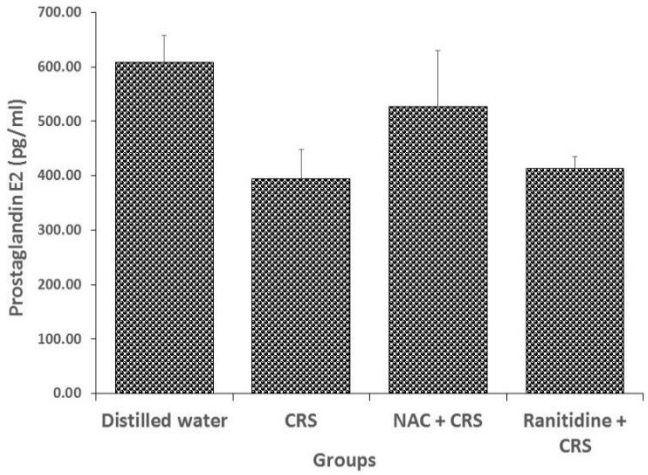


Figure 6: Effect of N-Acetyl-Cysteine on Prostaglandin E_2 Concentration in Wistar rats subjected to cold restraint stress. NAC = N-Acetyl Cysteine, n = 4

4. Discussion

Stress has been shown to be associated with altered homeostasis that may lead to oxidant-antioxidant imbalance. It was established that the pathogenesis of stress-induced gastric lesions includes the generation of reactive oxygen species (ROS) (Dragana *et al*., 2009). Antioxidants aid in many mechanisms, including the prevention of chain initiation, decomposition of peroxides, binding of transition metal ion catalysts and prevention of continued hydrogen abstraction. The present study investigated the modulatory role of N-Acetyl-Cysteine on gastric mucosal lesion and some biochemical changes in Wistar rats subjected to cold restraint stress. The preventive index of N-acetyl cysteine on CRS-induced ulcer was found to be 66.7 %. This suggests antioxidant properties of NAC might have contributed in gastroprotection in gastric mucosal lesions induced by CRS. This finding is in agreement with the work of Fadime *et al*. (2016) who investigated N-Acetyl cysteine gastro-protective and anti-inflammatory effects in experimental rat models. Results of the histological studies revealed slight mucosal necrosis and hyperplasia of inflammatory cells in the CRS group while only moderate necrosis was observed in the N-acetyl cysteine + CRS group, demonstrating the beneficial role of the N-acetyl cysteine in the protection of gastric mucosal lesions in the stress condition. The control group shows normal mucosal lining with slight necrosis. An increase in base-line

body weight was observed in the CRS group. This was in accord with finding of Peter *et al*., 2022 that Cold exposure drive weight gain and adiposity following suppression of brown adipose tissue and increase in sub-cutaneous fat mass

The effect of N-Acetyl-Cysteine on the MDA concentration in rats subjected to CRS Showed no significant change observed in the MDA concentration in all the treatment groups when compared to the control. Similarly, the effect of N-Acetyl-cysteine on SOD activity in rats subjected to CRS showed no significant change observed in the SOD activity in all the treatment groups when compared to the control. These changes could be short-lived, therefore unable 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 (Fadime *et al*., 2016) The effect of N-Acetyl-cysteine on INOS concentration in rats subjected to CRS showed a significant increase as observed in the INOS concentration in NAC + CRS (18.00 ± 0.42), Ranitidine + CRS (19.00 ± 0.40), when compared to the control, (14.23 ± 0.83), [F (7, 23) = 5.33; p = 0.001]. NAC produced a significant gastroprotection, similar to that observed in ranitidine (reference drug), indicating the role of NAC protective mechanisms in providing modulation of the iNOS signalling pathway by exerting potent antioxidant and anti-inflammatory effects. This finding was supported by a study conducted by Yasmin *et al*. (2022). Furthermore Lopez *et al*. (1991) found that oral administration of NAC protected the gastric mucosa against ethanol-induced gastric injury

The effect of N-Acetyl-cysteine on the PGE_2 concentration in rats subjected to CRS. Showed no significant change as observed in the PGE_2 concentration in all the treatment groups when compared with the control [F (7, 23) = 2.6; p = 0.06]. However higher PGE_2 concentration was observed in the NAC and ranitidine treated groups and the control when compared to the CRS group, with a particular trend towards significance.

5. Conclusions

In conclusion, based on the findings from our study, the high preventive index of N-acetyl cysteine on CRS induced ulcer was as a result of antioxidant properties of NAC which might have contributed to its' gastroprotection against gastric mucosal lesions. Acute administration of NAC significantly increased body weight of rats subjected to CRS.

Acknowledgements

The authors wish to thank TET Fund Nigeria for funding this research work through the Institutional Based Research Fund. We also wish to acknowledge Mr. Muktar Adamu Difa, Mr. Abubakar Ibrahim and Mr. Abubakar Bilyaminu Maikaho of the Animal House Facility of the Department of Human Physiology, Gombe State University for rearing of the animals

References

Akpinar, D., Yargiçoğlu1, P., Derin N., Alicigüzel, Y., and Ağar, A. (2008) The effect of lipoic acid on antioxidant status and lipid peroxidation in rats exposed to chronic restraint stress. *Physiological Research,* 57: 893-901

Ausama, A.J. (2015). Protective effect of N-acetylcysteine against ethanol-induced gastric ulcer: A pharmacological assessment in mice. *Journal of Inter cultural and Ethno pharmacology*, 4(2): 90–95 doi: 10.5455/jice.20150212103327

Dragana, D., Snežana, J. H., Slavica, R., Nevena, V., Radonjić., Nataša, D. P., Vesna, P., and Dušan, M. M (2009) Attenuation of cold restraint stress-induced gastric lesions by an olive leaf extract. *General Physiology Biophysics* **28**: 135–142

Elewa, K., Mohd Rafeeq, M., and Mohiuddin, M. (2012). Effect of vitamin D supplementation on cold restraint induced oxidative stress in rats. *Afri. J. of Pharm and Pharmacol*, 6(41): 2880-2883

Eqbal, M. A. D., Aminah, A., and Halimah, A.S. (2012). Lipid profile and antioxidant enzymes in normal and stressed rat fed with palm olein. *American Journal of Applied Sciences*, 9 (7): 1071-1078.

Fadime, A., Fehmi, O., Mesut, H., Elif, C., Ozlem, A., Zekai, H. and Ahmet C (2016) 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. *Journal of Cellular Biochemistry* 117:308–319 DOI 10.1002/jcb.2519

Guo, S., Gao, Q., Jiao, Q., Hao, W., Gao, X., and Cao, J. (2012). Gastric mucosal damage in water immersion stress: Mechanism and prevention with GHRP-6. *World J. of Gastroenterol*, 18(24): 3145-3155.

Güzel, C., Kurt, D., Sermet, A., Kanay, Z., Denli, O., and Canoruc, F. (1998). The effects of vitamin E on gastric ulcers and gastric mucosal barrier in stress induced rats. *Turkish Journal of Medical Sciences*, 28: 19-21.

Ishii, M., Shimizu, S., Nawata, S., Kiuchi, Y., and Yamamoto, T. (2000). Involvement of reactive oxygen species and nitric oxide in gastric ischemia reperfusion injury in rats: Protective effect of tetrahydrobiopterin. *Digistive Diseases and Sciences Journal*, 45: 93-98.

Kemidi, I. M. R., Vamshikrishna, P., Niroop, P., and BaikunthaPrusti, K. (2013). Protective effect of ginger against aspirin-induced ulcers in rats. *International Journal of Research in Pharmaceutical and Biomedical Sciences*, 4 (2): 597-606

Lopez, R.A., Tomwali, M.S., Henagan, J.M., Smith, G.S., and Miller, T.A. (1991). N. acetyl cysteine: protective agent or promoter of gastric damage? *Proceedings of the Society for Experimental Biology and Medicine*, 197(3):273-8 doi: 10.3181/00379727-197-43255

Mohamed, A. E. and Walaah, N. (2013). Involvement of ppar- γ activation in no-mediated gastric ulcer healing in rats. *Asian Journal of Pharmaceutical and Clinical Research*, (6) 2: 200-205

National Institute of Health (1985). Guide for the Care and Use of Laboratory Animals. *NIH Publication Number* 85-23. US Department of Health, Education and Welfare, Bethesda, MD

Peter, A., Jo, E. L. Irene, L., Ian, B., Ramyar, C., (2022). Cold exposure drive weight gain and adiposity following suppression of brown adipose tissue *International Journal of Molecular Science*, 23(3):1869 doi: 10.3390/ijms23031869

Ribeiro-Rama, A.C. , Fiqueiredo, I.V. , Veiga, F., Castel-Branco, M.M., Cabrita, A.M., and Caramona, M.M. (2009). Evaluation of gastric toxicity of indomethacin acid, salt form and complexed forms with hydroxypropyl-beta-cyclodextrin on wistar rats: histopathologic analoges. *Fundamental and Clinical Pharmacology*, 23(6): 747-55.

Sadau, Y., Adelaiye, A.B., Magaji, R.A., Ayo, J.O., Mabrouk, M.A., and Isa, A.I. (2015). Role of selenium and vitamin E on gastric mucosal damage induced by water-immersion restraint stress in Wistar Rats. *IOSR Journal of Pharmacy and Biological Sciences*, 10 (1): III 34-39

Shu, G., Qian, G., Qing, J., Wei, H. Xue, G. and Ji-Min, C. (2012). Gastric mucosal damage in water-immersion stress: Mechanism and prevention with GHRP-6. *World Journal of Gastroenterology*, 18 (24): 31453155.

Wong, D. Koo, M.W. Shin, V.Y. Liu, E.S. and Cho, C.H. (2002). Pathogenesis of nicotine treatment and its withdrawal on stress-induced gastric ulceration in rats. *European Journal of Pharmacology*, 434: 81-86

Yasmin, T., Mohamed A., Ibrahim, A., Naguib, B., Ali, A., Abo-Saif, A., Mohammed, H., Elkomy, C., Badrah, S., Alghamdi, D.E., and Wafaa, R. M. (2022) Role of ADMA/DDAH-1 and iNOS/eNOS signaling in the gastroprotective effect of tadalafil against indomethacin-induced gastric injury. *Biomedicine & Pharmacotherapy*, 150:113026 https://doi.org/10.1016/j.biopha.2022.113026