Method Article

Tocopherol Alleviates Exacerbation of Colitis Due to Cadmium Exposure in Wistar Rats: A Biochemical and Histological Study

ABSTRACT

Aims: This study investigated the effects of Tocopherol (vitamin E) on cadmium-exposed colitic rats.

Study design:A randomized control trial involving 40 male Wistar rats (180 ± 20g). Group 1: Nomal control; Group 2:Vitamin E-treated; Group 3: Colitis-inducedexposed; Group 4: Cadmium Chloride ($CdCl_2$); Group 5: Colitis + $CdCl_2$; Group 6: Colitis + vitamin E; Group 7: $CdCl_2$ + vitamin E; Group 8: Colitis + $CdCl_2$ + vitamin E.
Place and Duration of Study: Department of Physiology, Faculty of Basic Medical Sciences, Ladoke Akintola University of Technology, Oyo state Nigeria, between January 2024 and May 2024.

Methodology: The experiment lasted 30 days. Colitis wasinduced on day 23 using 2 mL/200g of 6% acetic acid intra-rectally. CdCl2 (50mg/kgb.w) was administered orally for 30 days, and vitamin E (100mg/kgb.w) lasted 7 days. Diarrhea score and body weight changes were assessed during the course of the experiment. Oxidative stress markers: Superoxidase dismutase (SOD) and Catalase (CAT); inflammatory markers: Myeloperoxidase(MPO) and Tumor Necrosis Factor alpha (TNF-α);neutrophils and lymphocyte counts and colon histology were also assessed. Data obtained was analyzed using one-way ANOVA, and Turkeys' post hoc test was used for comparison of inter-group differences. $P< 0.05$ was considered statistically significant.

Results: The study showed significant body weight decrease and increased diarrhea scores in cadmium-exposed and colitis + cadmium groups. SOD and CAT activities were significantly depleted, while MPO activity and TNF-α levels were markedly elevated compared to normal control and vitamin E-treated groups. Neutrophil count decreased while lymphocyte count increased in Cd-exposed and colitis + cadmium groups compared to the normalcontrol. Histological evaluation revealed inflammation and epithelial erosion in the colitis + cadmium group, with observed healing in vitamin E-treated groups.

Conclusion: The study concluded that vitamin E has the potential to mitigate the exacerbated effects of cadmium on acetic acid-induced colitis, attributed to its anti-oxidative and anti-inflammatory properties.

Keywords: Ulcerative colitis, vitamin E, cadmium, oxidative stress

1. INTRODUCTION

Ulcerative colitis (UC), a prevalent form of inflammatory bowel disease (IBD) is a chronic inflammatory condition that affects the gastrointestinal tract, particularly the distal colon and rectum [1]. The pathogenesis of this inflammatory condition remains elusive, yet its prevalence is attributed to a multifaceted interplay of factors such as geographical location, diet, genetics, immune response aberrations, and environmental triggers [2] [3] [4]. It is a relapsing disease that presents with symptoms such as abdominal discomfort, rectal bleeding, bloody watery stool and weight loss [5] [6].Previous studies have reported that ulcerative colitis is on the rise particularly in developing countries [7], affecting more than 3.5 million people worldwide and this presents as a major health challenge [8]. Cases of ulcerative colitis have been well-reported in the Western world [9][10][11] with significant variation in the incidence and prevalence in different countries. However, a study carried out by [7] on the epidemiology of IBD reported that a quite number of cases on UC are beginning to rise in some sub-

Saharan countries where it was initially uncommon. The continuing rise in UC occurrences has been attributed to industrialization which was associated with environmental contaminants ([12].

Cadmium (Cd) with atomic number 48, is a heavy toxic metal that accumulates in our surroundings as a result of industrialization. In a review conducted by Rafati et al. [13] Cd was identified as a potential global hazard since Cd compound poisoning can infect a person via routes such as, air, water, and food; which can result to negative impact on some organs and systems of the body]. A study conducted by Adegoke et al. [14] revealed that Cd toxicity could potentially aggravate the clinical course of UC. From literatures, it has been established that Cadmium poisoning can potentially result in susceptibility to ulcerative colitis [15]

Nutritional interventions, especially vitamins have been identified as an effective treatment of inflammatory conditions [16]; and one of such is vitamin E. It is a natural antioxidant known to modulate immune function and neutralize free radicals such as its potential to protect cell membrane against oxidation of polyunsaturated fatty acids (PUFA) [17]. The regulatory role of vitamin E in disease management was recently reviewed by Jiang et al. [18] they reported its potential effects of quenching reactive oxygen species (ROS) and mitigating inflammation. Several studies have also explained the benefits of consuming diets rich in tocopherols due to its anti-inflammatory property which helps in reducing the impact of oxidative stress on the immune system [19].

In managing ulcerative colitis, conventional treatments such as 5-amino salicylic acid (5-ASA), sulfasalazine, antibiotics, immunosuppressive agents, steroids (glucocorticoid), sulfasalazine and anti-tumor necrosis factor (TNF)-a (infliximab), usually cause serious side effects and sometimes, patients do not respond effectively to those drugs [20]. These kinds of treatments often lead to immune suppression, culminating in a compromised quality of life [21], and studies have established that individuals grappling with ulcerative colitis often exhibit micronutrient deficiencies, one of which is vitamin E [22]. This study was designed to investigate the protective role of vitamin E in managing UCin individuals exposed to cadmium toxicity.

2. MATERIAL AND METHODS

2.1 Chemicals and Supplement

The chemicals and supplement used in this study were of high analytical grade. Acetic acid (Eastman Chemical Company, United States), Cadmium chloride (Sigma chemical Co., India), Formalin (Hexion Inc. United States); Vitamin E (Gujarat pharmacaps, India)

2.2 Animals

Male Wistar rats were obtained from the Animal House of Ladoke Akintola University of Technology Ogbomoso Oyo State. They were acclimatized for two weeks prior commencement of the experiment. They were housed in ventilated plastic cages and kept under standard conditions (12hr light and 12hr dark period; temperature- 28-31^{o}C) with food (pelletized feed) and water *ad libitum* readily accessible to them. Ethical approval (ERCFBMSLAUTECH:020/01/2024) was obtained from the Ethics Research Committee (ERC) of Faculty of Basic Medical Science of the institution, and the animals were handled according to the International Guidelines on the Care and Use of Laboratory Animals in Research [23].

2.3 Experimental Design

Forty male Wistar rats weighing 180±20g were randomly distributed into eight groups (n=5) with each group receiving different treatments. Group 1 (Normal control): received distilled water for 30 days. Group 2 (Vitamin E): received 100mg/kg B.W of Tocopherol for 7 days. Group 3 (Colitis): received single intra-rectal administration of 2ml/200g B.W of 6% acetic-acid on the 23rd day. Group 4 (Cadmium): received 50mg/kg B.W of Cadmium Chloride ($CdCl_2$) for 30 days. Group 5(Colitis+Cadmium): colitis was induced after $CdCl_2$ exposure**.** Group 6 (Colitis+Vit E): received vitamin E for 7 days after 24 hrs post-colitis induction. Group 7 (Cadmium+Vit E): received vitamin E for 7 days after being exposed to $CdCl_2$ for 23 days. Group 8

(Colitis+Cadmium+Vit E): received $CdCl_2$ for 21 days, induced with colitis on the 23rd day, thereafter the rats were treated with vitamin E for 7 days

2.4 Induction of Colitis

After 21 days of cadmium exposure, rats in group 3, 5, 6 and 8 were left to fast for 24 hours prior to colitis induction. Rectal flushing was done to remove fecal remnant and then intra-rectal administration of 2ml/200g body weight of 6% Acetic acid (AA) was done with the aid of a rectal cannula, which was inserted into the colon through the rectum (8cm proximal to the anus) and this was retained for 55 seconds in a Trendelenburg position to avoid liquid extravasation as described by Doddaet al. [24].

2.5 Collection of Blood and Tissue Samples

The rats were anaesthetized with ketamine (50mg/kg) before the sacrifice. The heart was punctured through the retro orbitals plexus and blood samples were collected using a syringe and stored into Ethylene diamine tetra acetic acid (EDTA) bottles for haematological analysis. The colons (distal 6cm) were excised and carefully cleaned of blood stains and faecal content to avoid stretch and then stored appropriately for biochemical and histology analyses.

2.6 Colitis (Stool) Scoring

During the course of the experiment, the stool of the animals were observed and recorded daily by using the method of [25] with little modifications as follows: 0 – normal stool; 1 – normal stool with blood; 2 – loose stool without blood; 3 – loose stool with visible blood; 4 – blood watery stool.

2.7 Body Weight Changes

The weight of each rat was weighed and recorded daily throughout the duration of the experiment by using a digital weighing scale (Citizen Model MP2000). Before the sacrifice, they were also weighed and the change in weight was obtained using this formula: Weight Changes = final weight (before sacrifice) – initial weight (before colitis induction).

2.8 Biochemical Analysis

The proximal part of the colon was homogenized in phosphate buffer solution (PBS) and then centrifuged at a speed of 16,000rpm at 4°C for 20 minutes and the resultant supernatants were kept at 4°C for biochemical assay. Oxidative stress markers such as: Superoxide Dismutase (SOD), and Catalase (CAT) were analysed using calomeric method as described by [26][27] respectively. Inflammatory stress markers such as: Myeloperoxidase (MPO) and Tumor Necrosis Factor alpha (TNF-∝) as described by [28]were assayed using Enzyme-Linked Immunosorbent Assay (ELISA) kit (Elabscience Biotechnology Inc., U.S.A).

2.9 Histological Analysis

The distal part of the colonic tissue was rinsed in ice-cold normal saline and then fixed in 10% formalin solution. The tissue was removed after 24hrs and alcohol was added to induce dehydration. The tissue was then put in wax bath for infiltration/impregnation, which was set to run for twelve hours in order to harden/block. It was deparaffinised with xylene and chopped (using a microtome) into tiny pieces, no thicker than 4 mm and then stained with haematoxylin and eosin (H & E) to improve the contrast of the tissue structures for proper microscopic evaluation. The stained sections were assessed under a light microscope and then photomicrographs were taken at 100 magnifications for assessment of any histo-pathological alteration [29].

2.10 Statistical Analysis

The data was represented as mean ± SEM. With the aid of Graph pad Prism version 7.0 (Graph Pad statistical software. Inc. USA), one-way analysis of variance (ANOVA) was used to analyse the data. To compare results within different groups, Turkeys' post-hoc test was also employed.

3. RESULTS AND DISCUSSION

Table 1. Effect of Vitamin E on Body Weight Changes of Cadmium Exposed Colitic Wistar Rats

The body weight of cadmium group and colitis+ cadmium group was significantly decreased when compared with the control group. The decrease in body weight might be due to a decline in the absorption of lipids and proteins which could be caused by cadmium exposure. Studies have shown that cadmium, when inhaled or ingested can compromise metabolism resulting in low weight gain [14] [30].

Groups	Final Weight(g)	Initial Weight(g)	Weight Changes (g)
Control	213.0 ± 4.669	158.2 ± 2.596	54.8 ± 2.073
Vitamin E	149.4 ± 3.600	115.0 ± 3.066	34.4 ± 0.534
Colitis	210.8 ± 3.891	181.2 ± 3.023	29.6 ± 0.868
Cadmium	172.0 ± 3.033	191.2 ± 3.760	-19.2 ± -0.727**a**
Colitis + cadmium	183.2 ± 4.944	175.6 ± 6.120	7.6 ± -1.176[b]
Cadmium + Vitamin E	200.2 ± 9.609	184.0 ± 13.87	16.2 ± -4.261
Colitis + Vitamin E	169.2 ± 14.11	152.8 ± 11.97	16.4 ± 2.14
Colitis + cadmium Vitamin E	194.6 ± 5.528	176.8 ± 5.526	17.8 ± 0.002

Table 1: ([a]represents significant decrease at *P* = .05 when compared with Control group. [b]represents significant decrease at *P* = .05 when compared with colitis + cadmium + vitamin E group).

Table 2. Effect of Vitamin E on Diarrhea Score of Cadmium Exposed ColiticWistar Rats

Cadmium exposure increased the stool consistency score in the colitis + cadmium group when compared with the colitis and cadmium groups. Inflammatory responses of acetic acid-induced colitis, in addition to cadmium's existing effect might have increased oxidative stress in the GIT of the rats, thereby reducing colitis healing (as measured by stool consistency score) and this findings corroborates with the work of Adegoke et al. [31].

Groups	Diarrhea Score
Control	0.00 ± 0.00
Vitamin E	0.00 ± 0.00
Colitis	0.60 ± 0.25
Cadmium	0.20 ± 0.20
Colitis + cadmium	1.50 ± 0.87[a]
Cadmium + vitamin E	0.20 ± 0.20
Colitis + vitamin E	0.40 ± 0.40
Colitis + cadmium + vitamin E	0.80 ± 0.37

Table 2: ([a]represents significant decrease at $P = .05$ when compared with colitis and cadmium group).

Table 3 Effect of Vitamin E and Cadmium on Neutrophil and Lymphocyte Counts

Neutrophil and lymphocyte ratio is an index used in determining disease progression particularly in inflammatory conditions [32]. When compared to the control group, there was a significant increase in neutrophil count in cadmium group and colitis + cadmium group and significant decrease in lymphocyte count in cadmium group when compared with the control and vitamin E group. The study conducted by Adegoke et al.[14] iterated that this could be due to the continuous activation of inflammatory cells caused by cadmium.

NLR	Control	Vit E	Colitis	Cadmium (Cd)	Colitis + Cd	Colitis + Vit E	Cd + Vit E	Colitis + Cd + Vit E
NEU	31.20 ± 2.15	28.50 ± 5.05	37.75 ± 4.00	54.00 ± 3.83[a]	58.67 ± 3.48[b]	28.67 ± 3.66	42.25 ± 1.54	39.25 ± 3.90[c]
LYM	65.80 ± 1.28	67.00 ± 4.35	63.40 ± 4.35	39.40 ± 5.84[a]	49.40 ± 6.15[b]	69.00 ± 3.48	53.40 ± 3.54	63.40 ± 79.40[c]

Table 3: (significant increase in neutrophil count ($P = .05$) and decrease in lymphocyte count ($P<.01$) of cadmium group and colitis + cadmium group, when compared with colitis + cadmium +vitamin E group)

Figure 1. Effect of Vitamin E on Superoxide Dismutase (SOD) Activity of Cadmium Exposed Colitic Wistar Rats

Superoxide dismutase (SOD) is known to be the first line of defense in GIT against free radicals by attenuating oxidative stress associated with ulcerative colitis In this study, the depletion of SOD activities in colitis + cadmium group illustrates how cadmium exposure and acetic acid-induced colitis work together to weaken endogenous antioxidant defenses. Cadmium, which is a heavy toxic metal, interferes with antioxidant enzymes and causes reactive oxygen species (ROS) to be produced; and this upsets cellular redox homeostasis. Earlier research of Valko et al. [33] revealed that cadmium-induced ROS production not only diminishes SOD activity but also triggers a cascade of events that exacerbate tissue damage and inflammation. On treatment with vitamin E, SOD concentration was improved in the Colitis + Cd + Vit E group when compared to the Colitis + Cd group. Studies have shown that vitamin E can interrupt lipid peroxidation by scavenging lipid-derived radicals, which could contribute to the preservation of SOD activity. Additionally, the synergy between Vitamin E and other endogenous antioxidants, such as glutathione, could collectively augment SOD activity [35] [41].

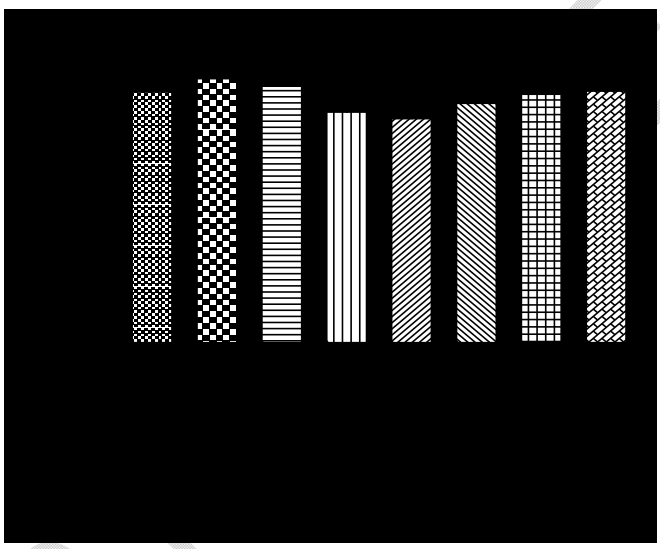


Figure 1: (data were presented as mean ± SEM of 5 rats[a] represents significant decrease at *P* = .05 when compared with control group. [b]represents significant decrease at *P*= .05 when compared with control group. [c]represents significant decrease at *P*< .01 when compared with the colitis group. [d]represents significant increase at *P*< .01 when compared with colitis + cadmium group).

Figure 2 Effect of Vitamin E Catalase Activity of Cadmium Exposed Colitic Wistar Rats

Catalase, which is also an essential endogenous antioxidant, operates by using hydrogen peroxide detoxification to stop the build-up of ROS [34]. It revealed depleted catalase activity in the colitis, cadmium, and colitis + cadmium groups when compared with vitamin E treated groups as shown in Figure 2. This finding corresponds with previous studies, proving vitamin E's ability to mop up free radicals from the cytosol of the mitochondria [35].

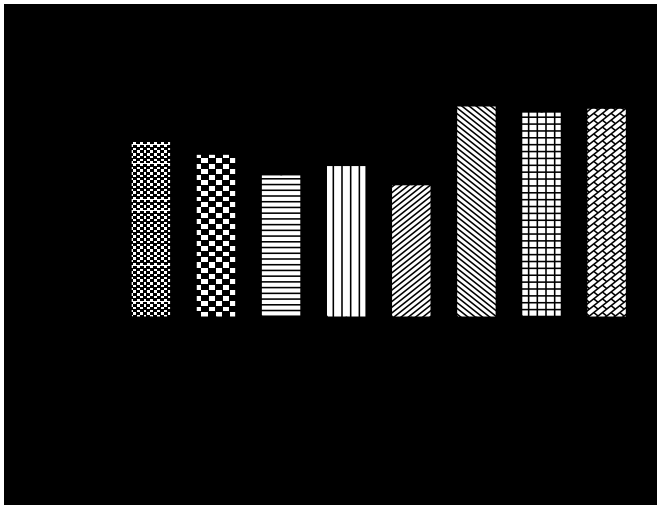


Figure 2: (data were presented as mean ± SEM of 5 rats[a,b]represents significant decrease at *P* = .05 when compared with control group.[c]represents significant decrease at *P* = .05 when compared with the colitis + cadmium + vit E group). [d]represents significant increase at *P* = .05 when compared with the colitis + cadmiumgroup).

Figure 3 Effect of Vitamin E on Myeloperoxidase (MPO) Activity of Cadmium Exposed Colitic Rats

Myeloperoxidase (MPO) is a heme-containing enzyme predominantly expressed in neutrophils, and plays a pivotal role in catalysing the formation of ROS and contributing to tissue damage [36]. Cadmium exposure led to elevated MPO activity in the cadmium and colitis + cadmium groups when compared with the control group, which were elicited by pro-inflammatory milleu resulting in the infiltration of neutrophils in the cellular level. The observed up-regulation of MPO could be attributed to the synergistic interaction between colitis-induced tissue damage and cadmium-induced inflammation. In contrast, MPO activity was significantly decreased in the vitamin E treated groups. This result accentuates with previous studies of the potential ameliorative role of vitamin E in mitigating neutrophils recruitment in the colonic mucosa [35][37].

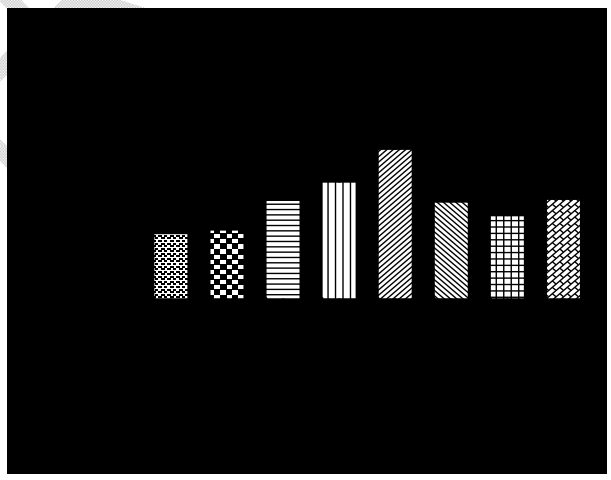


Figure 3: (data were presented as mean ± SEM of 5 rats [a] represents significant increase at *P* = .05 when compared with control group. [b]represents significant increase at *P*< .001 when compared with the control group. [c]represents significant increase at *P* = .05 when compared with colitis induced group. [d]represents significant decrease at *P*<.05 when compared with vitamin E treated groups).

Figure 4 Effect of Vitamin E on Tumour Necrosis Factor-Alpha (TNF-α) of Cadmium Exposed Colitic Wistar Rats

Inflammation, though a physiological response lies at the heart of numerous pathological conditions, including inflammatory bowel diseases and exerts a multi-layered influence on cellular and systemic homeostasis. Tumor necrosis factor (TNF-α), a potent pro-inflammatory cytokine holds a central role in modulating immune response. In this study, TNF-α activity was markedly increased in the colitis + cadmium group when compared with the control group, colitis group and cadmium group. This may be possibly due to cadmium potential to activateMitogen-Activated Protein Kinase(MAPK)pathways, thereby exacerbating inflammation on the colonic mucosa of the rats [38][39]. The anti-inflammatory property of vitamin E was able to suppress the pro-inflammatory cytokines in the colonic tissues and this findings is consistent with the work of Kini et al. [37].

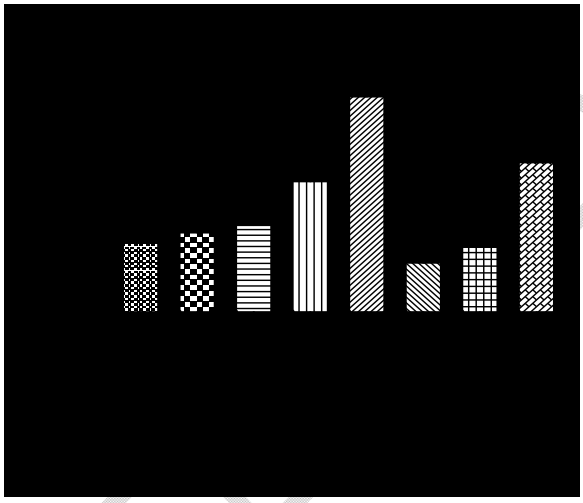
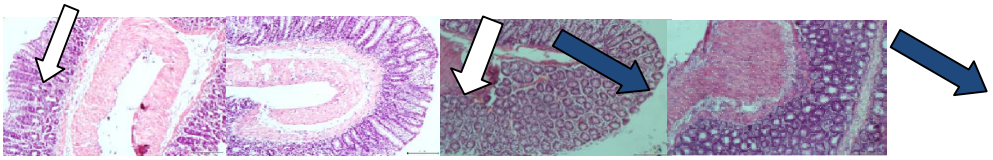


Figure 4: (data were presented as mean ± SEM of 5 rats[a] represents significant increase at *P*< .001 when compared with control group. [b]represents significant increase at *P* < .01 when compared with colitis-induced group. [c]represents significant increase at *P*<.05 when compared with Cd-exposed group).

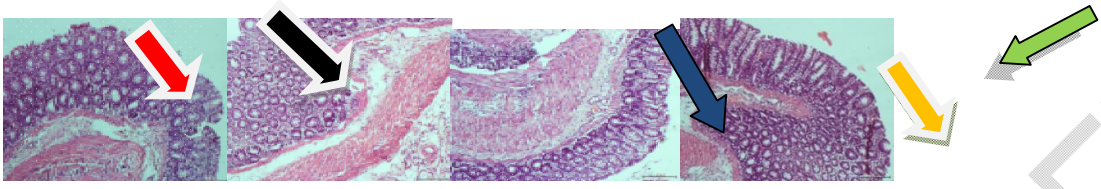
Figure 5: Histological Assessment

Histological analysis revealed that, the combined effect of colitis and cadmium eroded the epithelium and inflamed the colonic tissues, thereby slowing the healing process. Comparing the intact structure of crypts in the control and vitamin E groups to the colitis + cadmium group, cross-sections of the colonic tissue samples revealed multiple instances of inflammatory infiltration, particularly into the lamina propria and submucosa, deterioration of crypt architecture, and formation of crypt abscess. This could be as a result of cadmium's on-going stimulation of colonic inflammatory cells over time. Similar result was reported by Zalups and Ahmad[40] who found that oral administration of cadmium compounds resulted in colonic epithelial desquamation. Healing process was observed in colitis + cadmium group treated with vitamin E. This led to the reduction in the wide spread of inflammatory reaction and tissue damage brought on by colitis and cadmium as seen in plate 8.

The mucosal crypts and lamina propria of the control and vitamin E group appears normal (white arrow). There is mild inflammation of the mucosal crypts and lamina propria of the colitis group, cadmium group and cadmium+vit E group (blue arrow). The mucosal crypts, lamina propria and submucosa of the colits+Cd group shows chronic inflammation composed of large lymphoid follicles and a disrupted mucosal crypt architecture (red arrow). The lamina propria of colitis+vit E also appears inflamed mildly (black arrow). In colitis+cadmium+vit E group, there is mild inflammation on the lamina propria (yellow arrow), and healing already taking place on some part of the lamina propria (green arrow) (Plates 1-8).



Control Vitamin E Colitis Cadmium



Colitis+Cd Colitis+Vit E Cd+Vit E Colitis+Cd+Vit.E

Photomicrographs: (histological assessment of the mucosa of cadmium exposed colitic rats H&E ×100 magnification)

4. CONCLUSION

This study revealed the exacerbated effect of cadmium on the colonic mucosa of Wistar rats. From this study, vitamin E treatment proved to be an excellent free radical scavenger by counteracting lipid peroxidation and modulating cytokine expression. Vitamin E improved the redox status by ameliorating oxidative stress, inhibiting neutrophils infiltration, down-regulated pro-inflammatory cytokines, and reversing the histo-pathological damages on the colonic mucosa. Due to its anti-oxidative and anti-inflammatory properties, this accentuates its potency as a therapeutic agent against the combined challenges of colitis and cadmium exposure.

ETHICAL APPROVAL

All authors hereby declare that "Principles of Laboratory Animal Care" (NIH Publication No. 85-23, revised 1985) were followed. All experiments have been examined and approved by the appropriate ethics committee (ERCFBMSLAUTECH:020/01/2024)

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