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Full-Length Research Article

Tocopherol Enhances the Antioxidant Defense System and Histomorphometric Parameters in the Gastrointestinal Tract of Rats Treated with Sodium Arsenite

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Summary: Arsenic compromises the gastrointestinal integrity and function via the body's anti-oxidative system breakdown. Hence, this study aimed to investigate the effects of tocopherol on redox imbalance and histoarchitectural alterations in rats' gastrointestinal tract exposed to sodium arsenite. Sodium arsenite (10mg/Kg) and graded doses of tocopherol were administered for four weeks concurrently (per oral) to experimental rats assigned to different groups as follows i: Control normal saline (1 mg/Kg); ii: Rats treated with Sodium arsenite (10 mg/Kg); iii: Rats treated with 100 mg/kg tocopherol + Sodium arsenite; iv: Rats treated with 300 mg/kg tocopherol + Sodium arsenite; v: Rats treated with Olive oil (0.5ml/Kg) only; vi: Rats treated with Olive oil and 100 mg/kg tocopherol; vii: Rats treated with Olive oil and 300 mg/kg tocopherol. Redox status assay was done in homogenized samples by spectrophotometry. Parietal cell mass and mucous cell density (stomach), villus height and crypt depth (ileum), goblet cells count, and crypt depth (colon) were evaluated by histomorphometry. Inflammatory cells infiltration was also assessed using a semi-quantitative procedure. Sodium arsenite caused a significant increase in Malondialdehyde and Myeloperoxidase but, decreased Superoxide dismutase, Catalase, Nitric oxide, Glutathione peroxidase, Glutathione, and Glutathione-S-Transferase. Tocopherol treatment reversed the changes ($p<0.05$) though not largely dose-dependent. Furthermore, tocopherol annulled sodium arsenite-induced increase in parietal cell mass and decrease in mucous cell density in the stomach, decrease in villus height and villus height/crypt depth ratio in the ileum, and decrease in goblets cells and increase in crypt depth in the colon. Moreover, activated inflammatory cell infiltration by sodium arsenite was mitigated by tocopherol. Sodium arsenite provokes not only marked inflammatory cellular infiltration but a focal loss of glands, hyperplasia of crypts, atrophic villi, and hypertrophy of Peyer's patches in the intestines, which are all lessened with tocopherol treatment. These findings underscore the anti-oxidative properties of tocopherol as a potent dietary factor against sodium arsenite toxicity in the gastrointestinal tract.

Keywords*: Tocopherol, arsenic, stomach, ileum, colon*

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INTRODUCTION

Arsenic (AS) is one of the most abundant toxic metals to which approximately 200 million humans are exposed particularly in developing countries (WHO,2012). More people are being increasingly predisposed due to the periodic societal adjustments in the processing and quality of foodstuff. For example, plants, an intermediate energy level in the food chain pathway preceding human exposure have been reported to bioconcentrate AS in the form of fruits, vegetables, and beverages (Chen et al.,2011). There is evidence of AS water contamination in many countries of the world such as India (Pradhan and Kumar 2014), Australia (Kiddee et al., 2014), China (Chau et al., 2015), Vietnam (Smith et al., 2000), Burkina Faso (Somé et al., 2012), and Ghana (Asante et al., 2012) particularly through electronic wastes. The recent incorporation of rice in baby diets may add the latter to the growing list of AS exposed subjects. Roxarsone, a dietary supplement used to stimulate growth in the poultry industry contains organic arsenic (Nachman et al., 2013). In Nigeria, AS has been reported to contaminate the soil (Anselm et al., 2021), water (Karkarna and Matazu 2021), plants (Olafisoye et al., 2013) dust (Ibe et al., 2018), and even blood/serum (Popoola et al., 2019) owing to poor environmental management.

Most heavy metals are impervious to digestive activities. In the case of AS, intestinal absorption follows the ingestion of inorganic AS polluted water and is thereafter sequestered into bio accumulative tissues via circulation (Kitchin and Kirk 2001). This process is accompanied by AS-induced toxicity such as ocular burning sensation, body weakness, lower limbs swelling, pulmonary distress, hepatic fibrosis, neurological disorder, and non-specific organ cancer (Singh et al., 2011) as the toxicosis traverses several areas of the body (Szymanska-Chabowska et al., 2002). One of the

notable pathophysiological disease mechanisms for which AS is identified including carcinogenesis, is induction of oxidative stress Chih-Hung et al., 2010) through the generation of Reactive Oxygen Species (ROS) which in turn disrupt the structure and function of important biomolecules such as protein, lipid, and DNA (Mo et al., 2006) thereby altering biochemical status (Yousef et al., 2008). AS and its intermediates in the process of their systemic clearance in the body are bio-transformed into methylated products that are not only bioavailable but are also cytotoxic (Petrick et al., 2000), genotoxic (Mass et al., 2001) and are biomolecular inhibitor of several enzymes (Drobná et al., 2003) and signaling pathway disruptors (Stýblo et al., 2002). Due to the ingestive nature of human exposure to AS (Chiocchetti et al., 2019), the gastrointestinal system is the prime target for its toxicity with such symptoms as abdominal discomfort, vomiting, and most commonly diarrhea. AS exposure has been reported to affect intestinal homeostasis, disrupt the mucosal layer (Chiocchetti et al., 2018), and predispose the gastrointestinal tract to ulceration (Adebayo-Gege et al., 2018).

Tocopherol commonly referred to as Vitamin E is a fat-soluble diet-derived natural antioxidant known to be well absorbed in the gastrointestinal tract (Traber and Arai 1999) as it mixes well with micelles and ferries through the enterocytes into extra-gastrointestinal organs (Reboul 2017). It has been well established that vitamin E is a major antioxidant in cellular membranes preventing peroxidation of cell membrane (Jiang 2014) by scavenging peroxyl, oxygen, and superoxide anion radicals. Though its potency as an exogenous gastroprotective and therapeutic factor has been mentioned (Kamisah et al., 2014), the level of knowledge on the mitigating effect of tocopherol on AS exposed gastrointestinal system is still incompletely understood. But it is established that the etiology of AS-induced gastrointestinal injury is biochemically mediated. Hence, this study investigates arsenite-induced oxidative stress and histoarchitectural changes in stomach, ileum, liver, and colon tissues, and the possible amelioration by tocopherol using laboratory rats.

MATERIALS AND METHODS

Animals and Experimental Design: Thirty-five (35) male Wistar rats weighing 100–120 g were obtained from the College Central Animal Facility of College of Health Sciences, Osun State University, Osogbo, Nigeria. The animals were kept in plastic cages in a well-ventilated animal house. They were fed with commercial rat chow and water *ad libitum*. Handling and care of the experimental animals were in strict accordance with the criteria outlined in the Current Animal Care Regulations and Standards approved by the Institute for Laboratory Animal Research (Guide for Care and Use of Laboratory Animals in Biomedical and Behavioral Research). The institution's Animal Ethics and Research Committee approved the experimental protocols (Protool I.D: UNIOSUNHREC/2021B/003).

Animals were randomly divided into seven groups of five animals each as follows- i: Control rats receiving normal saline (1 mg/Kg); ii: Rats treated with Sodium arsenite (10 mg/Kg); iii: Rats treated with tocopherol (100 mg/kg; peroral), followed by administration Sodium arsenite; iv: Rats treated with tocopherol (300 mg/kg; per oral), followed administration of Sodium arsenite; v: Rats treated with Olive oil (0.5ml/Kg) only; vi: Rats treated with Olive oil and tocopherol (100 mg/Kg); vii: Rats treated with Olive oil and tocopherol (300 mg/Kg).

Administration of sodium arsenite and tocopherol was done for four weeks concurrently. Rats were euthanized 24 h after the last administration, then, the stomach, ileum, and colon were removed. The stomach was cut open along the greater curvature while the intestines were opened along their entire lengths to wash off the debris. Small portions of the different segments of the gastrointestinal tract were cut for histological processing and analysis. The remaining portions were rinsed and homogenized using 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% KCl. The homogenate was centrifuged at 4°C for 10 minutes at a speed of 10,000x g, and the supernatant thereof was kept at -8ºC and used for the estimation of the biochemical parameters.

Drugs and Chemicals: Tocopherol capsules (Vitamin E) were purchased from a local Pharmacy duly registered by the Pharmacists' Council of Nigeria (PCN). Sodium arsenite, adrenaline, glutathione, 5,5'-dithiobis-2-nitrobenzoic acid, hydrogen peroxide (H_2O_2), thiobarbituric acid, and trichloroacetic acid, were purchased from Sigma Chemical (St. Louis, MO). All other reagents used were of analytical grade and were obtained from British Drug houses.

Biochemical Assays: MDA concentration as an index of lipid peroxidation was quantified according to the method described by Varshney and Kale (1990). Myeloperoxidase (MPO) activity was determined by the method of Xia and Zweier (1997). Superoxide dismutase (SOD) assay was carried out by the method of Misra and Fridovich (1972), Catalase (CAT) activity using H_2O_2 as substrate was measured by the method of Claiborne (1995) while Nitric Oxide (NO) content was quantified as previously described by Olaleye *et al*. (2007). Glutathione (GSH) concentration was determined using the method of Jollow *et al*. (1974). Glutathione peroxidase (GPX) activity was measured by the method of Rotruck *et al*. (1973). Glutathione S-transferase (GST) was estimated by the method of Habig *et al*. (1974) using 1-chloro-2,4-dinitrobenzene as substrate.

Histopathological Studies: Samples of the stomach, ileum, and colon were collected from each animal and fixed in 10% neutral formalin for 48–72 h. The tissue samples were processed in an automatic tissue processor – dehydrated and embedded in paraffin wax. Sections were cut at a thickness of 4 μm and stained with hematoxylin and eosin for histopathology. Standard glass microscope slides were used for mounting.

Histomorphometry: Inflammatory cells infiltration assessment was done subjectively using the scale: 0 = no infiltration; l = very mild infiltration; 2 = mild infiltration; 3 = moderate infiltration; 4 = marked infiltration. Parietal cell mass and mucous cell density were calculated as described by Ajeigbe *et al*., (2014) as the number of cells per mm^2 multiplied by the thickness of the glandular layer.

In the ileum and colon, villous height, villous width, and crypt depth were measured and assessed. At light microscopy (10 × objective lens), intact and 10 well-oriented villi and crypts from each intestinal section of each animal were randomly selected for measurement. The villus length was measured from the villus-crypt junction to the villus tip and the crypt depth from the crypt base to the villus-crypt junction. Using the same villus and crypt columns, the number of goblet cells was determined, expressed per 100 enterocytes. The villus/crypt ratio was calculated by dividing the villus height by crypt depth (Van Zuidewijn *et al*., 1992). The samples were evaluated, and measurements were performed with Olympus CX43 microscope with a color digital camera connected to a computerized image analysis system (Image Pro, USA).

Statistical Analysis

Data are presented as mean ± SEM and subjected to one-way analysis of variance (ANOVA) and Newman-Keul posthoc test using the Graphpad Prism version 6.0 for Windows from GraphPad Software, San Diego, CA, USA. Values of $p < 0.05$ were regarded as significant

RESULTS

Effect of tocopherol on lipid peroxidation induced by sodium arsenite in the gastrointestinal tissues of rats:

The ameliorative effect of tocopherol on sodium arsenite-induced lipid peroxidation is shown in Figures 1a, 2a, 3a and 1b, 2b, 3b. Sodium arsenite significantly increased malondialdehyde (MDA), an important marker of lipid peroxidation, when compared with the normal saline (control) group ($p<0.05$). However, treatment with 100 mg/Kg and 300 mg/Kg tocopherol reduced the elevation observed in the sodium arsenite alone group ($p<0.05$). Likewise, myeloperoxidase (MPO) activity was reduced in the stomach, ileum, and colon tissues by tocopherol in the sodium arsenite rats ($p<0.05$).

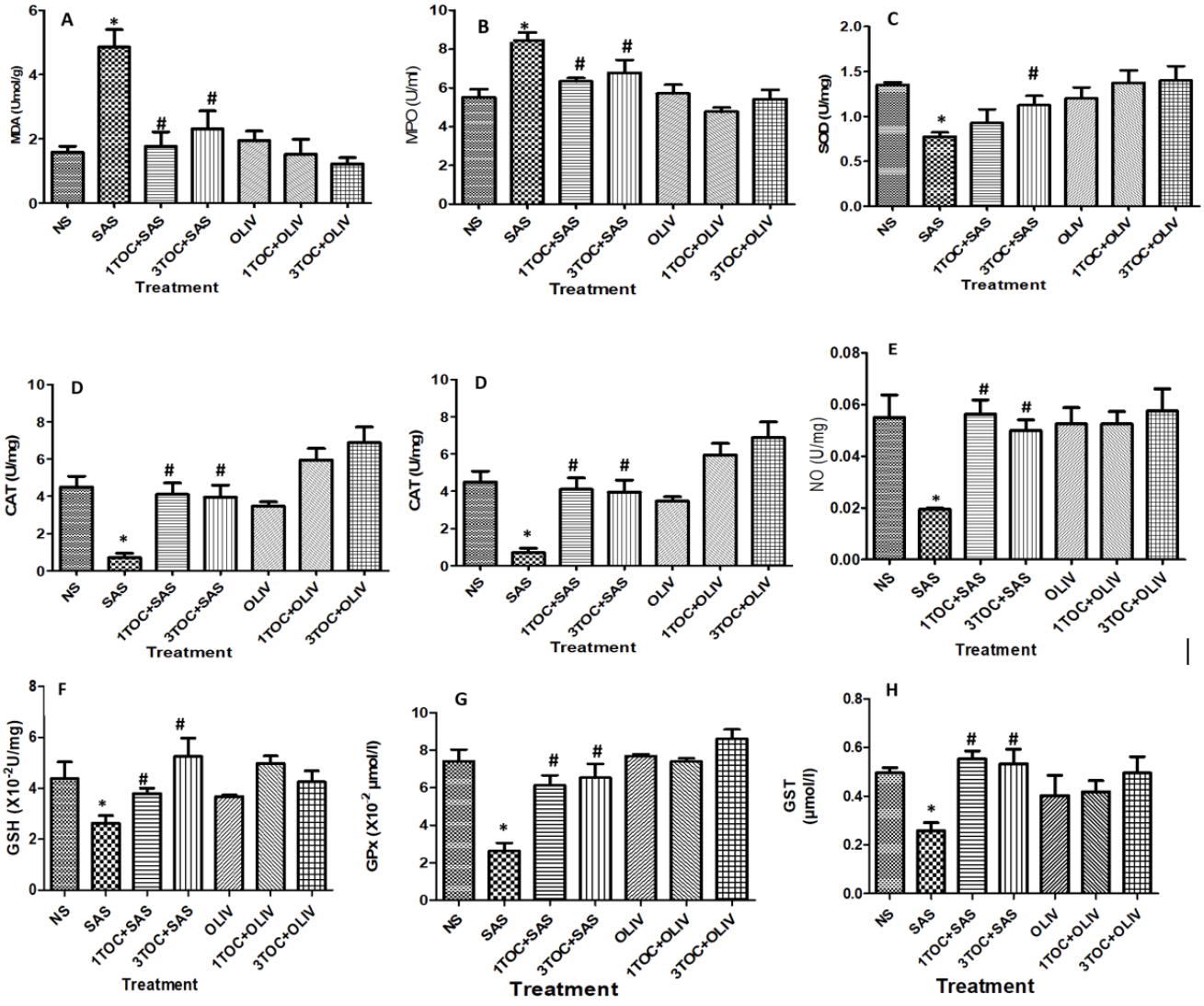

Figure 1:

Redox status in normal and sodium-arsenite exposed stomach tissue upon treatment with tocopherol. (A); MDA=Malondialdehyde, (B); MPO= Myeloperoxidase, (C); SOD= Superoxide dismutase, (D); CAT= Catalase, (E); NO=Nitric Oxide, (F); GSH=Glutathione (G); GPx=Glutathione Peroxidase, (H); GST=Glutathione S-Transferase**.** NS=Normal saline, SAS=Sodium-arsenite, 1TOC+SAS= 100 mg/kg Tocopherol + Sodium arsenite, 3TOC+SAS= 300 mg/kg Tocopherol + Sodium-arsenite, OLIV=Olive oil, 1TOC+OLIV= 100 mg/kg Tocopherol + Olive oil, 3TOC+OLIV= 300 mg/kg Tocopherol + Olive oil**.** Each bar represents means value and standard error, n=5. Significant difference is shown as *$p<0.05$ when compared with the control (NS), and $^{\#}p<0.05$ when compared with the sodium-arsenite treated (SAS) only**.**

In the sodium arsenite alone group, lipid peroxidation was significantly enhanced by increased myeloperoxidase activity when compared with the normal saline (control) group ($p<0.05$). The groups treated with tocopherol alone showed no significance when compared with the normal saline group ($p>0.05$) either in MDA or MPO activity.

Effect of tocopherol on antioxidant systems of the gastrointestinal tissues exposed to sodium arsenite:
Sodium arsenite exposure reduced all the antioxidant enzymes activities viz: Superoxide dismutase (SOD), Catalase (CAT), Glutathione (GSH), Glutathione peroxidase (GPx) and Glutathione S-transferase (GST) in the stomach (Figure 1c, d, f, g, h), ileum (Figure 2c, d, f, g, h) and colon (Figure 3c, d, f, g, h) tissues when compared with the normal saline (control) group ($p<0.05$). However, 100 mg/Kg and 300 mg/Kg tocopherol increased the activities of all the antioxidants in the gastrointestinal tissues when compared with the sodium arsenite alone treated group. Similarly, an erstwhile reduction in gastric, ileal, and colonic nitric oxide by sodium arsenite was reversed with tocopherol treatment ($p<0.05$) (Figure 1e, 2e, 3e). The groups treated with tocopherol alone showed no significance when compared with the normal saline group ($p>0.05$) either in the nitric oxide or antioxidant enzymes activities.

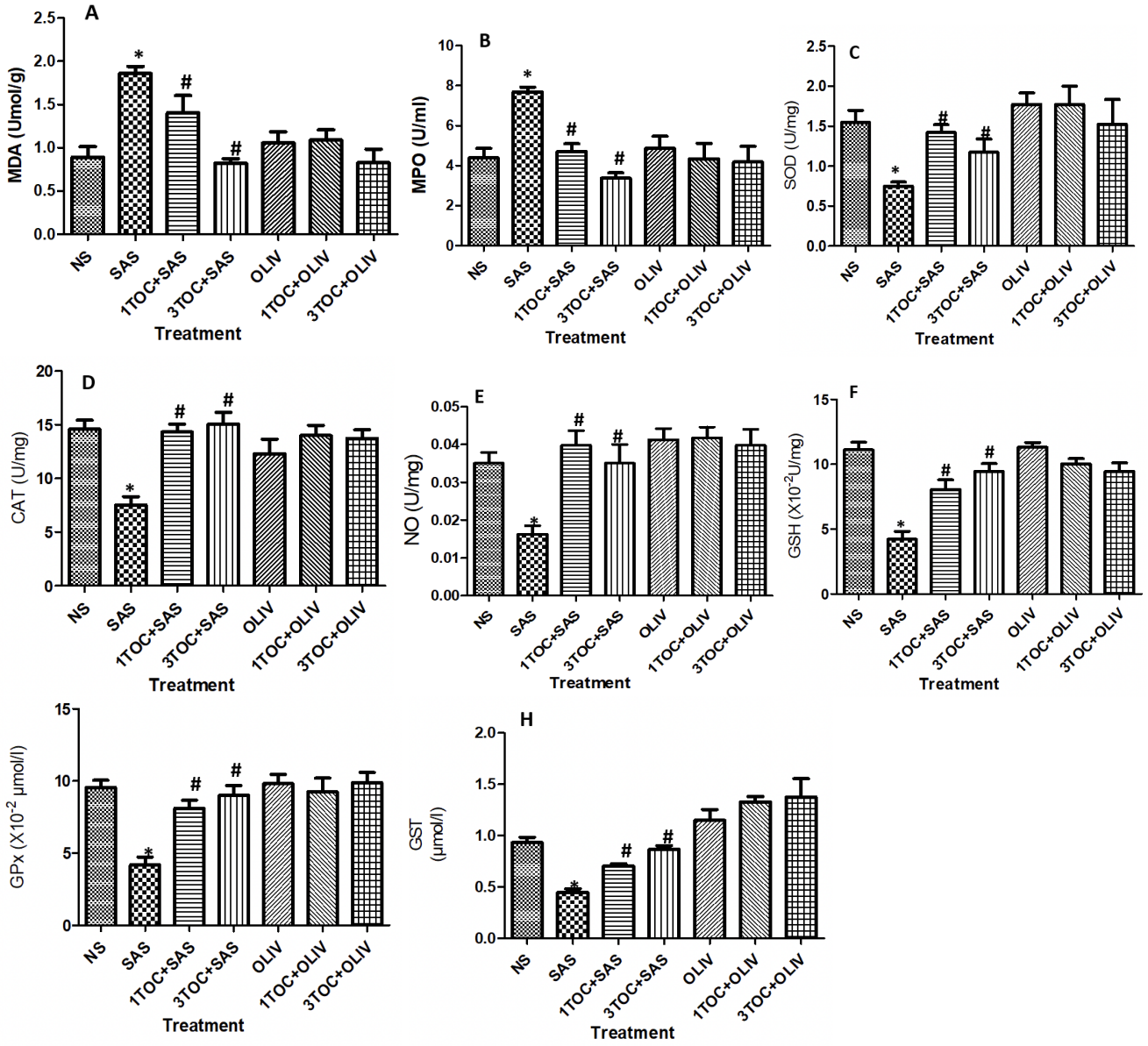

Figure 2:
Redox status in normal and sodium-arsenite exposed ileum tissue upon treatment with tocopherol. (A); MDA=Malondialdehyde, (B); MPO= Myeloperoxidase, (C); SOD= Superoxide dismutase, (D); CAT= Catalase, (E); NO=Nitric Oxide, (F); GSH=Glutathione (G); GPx=Glutathione Peroxidase, (H); GST=Glutathione S-Transferase**.** NS=Normal saline, SAS=Sodium-arsenite, 1TOC+SAS= 100 mg/kg Tocopherol + Sodium arsenite, 3TOC+SAS= 300 mg/kg Tocopherol + Sodium-arsenite, OLIV=Olive oil, 1TOC+OLIV= 100 mg/kg Tocopherol + Olive oil, 3TOC+OLIV= 300 mg/kg Tocopherol + Olive oil**.** Each bar represents means value and standard error, n=5. Significant difference is shown as *$p<0.05$ when compared with the control (NS), and $^{\#}p<0.05$ when compared with the sodium-arsenite treated (SAS) only

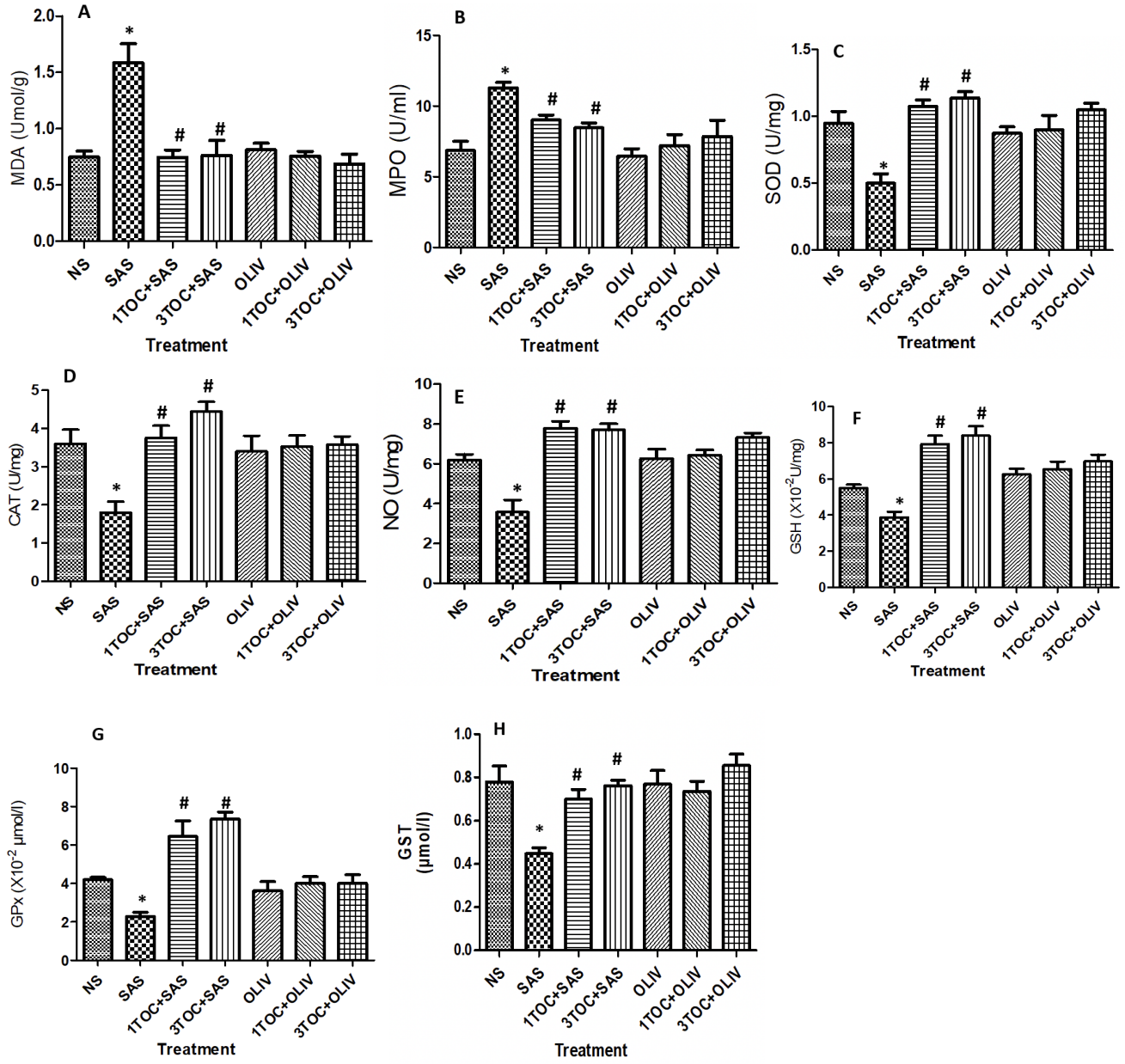

Figure 3:
Redox status in normal and sodium-arsenite exposed colon tissue upon treatment with tocopherol. (A); MDA=Malondialdehyde, (B); MPO= Myeloperoxidase, (C); SOD= Superoxide dismutase, (D); CAT= Catalase, (E); NO=Nitric Oxide, (F); GSH=Glutathione (G); GPx=Glutathione Peroxidase, (H); GST=Glutathione S-Transferase**.** NS=Normal saline, SAS=Sodium-arsenite, 1TOC+SAS= 100 mg/kg Tocopherol + Sodium arsenite, 3TOC+SAS= 300 mg/kg Tocopherol + Sodium-arsenite, OLIV=Olive oil, 1TOC+OLIV= 100 mg/kg Tocopherol + Olive oil, 3TOC+OLIV= 300 mg/kg Tocopherol + Olive oil**.** Each bar represents means value and standard error, n=5. Significant difference is shown as *p<0.05 when compared with the control (NS), and #p<0.05 when compared with the sodium-arsenite treated (SAS) only.

Table 1:
Effects of graded doses of tocopherol on sodium arsenite-induced toxicity in the rat's stomach: Parietal cell mass, mucous cell count, and Inflammatory cell infiltration

	Parietal Cell Mass	Mucous Cell Count	Inflammatory Cell Infiltration score
NS	8.60±0.40	13.20±0.37	0.60±0.20
SAS	15.00±0.30*	6.20±0.40*	2.20±0.20*
1TOC+SAS	10.20±0.60#	8.80±0.30#	1.20±0.20#
3TOC+SAS	10.40±0.50#	10.60±0.40#	1.20±0.20#
OLIV	8.30±0.37	12.00±0.30	0.60±0.20
1TOC+OLIV	9.20±0.58	14.80±0.35	0.60±0.20
3TOC+OLIV	8.80±0.37	13.40±0.50	0.60±0.20

The value represents means value and standard error, n=5. A significant difference is shown as *p<0.05 when compared with the control (NS), and #p<0.05 when compared with the sodium-arsenite treated (SAS) only**.**

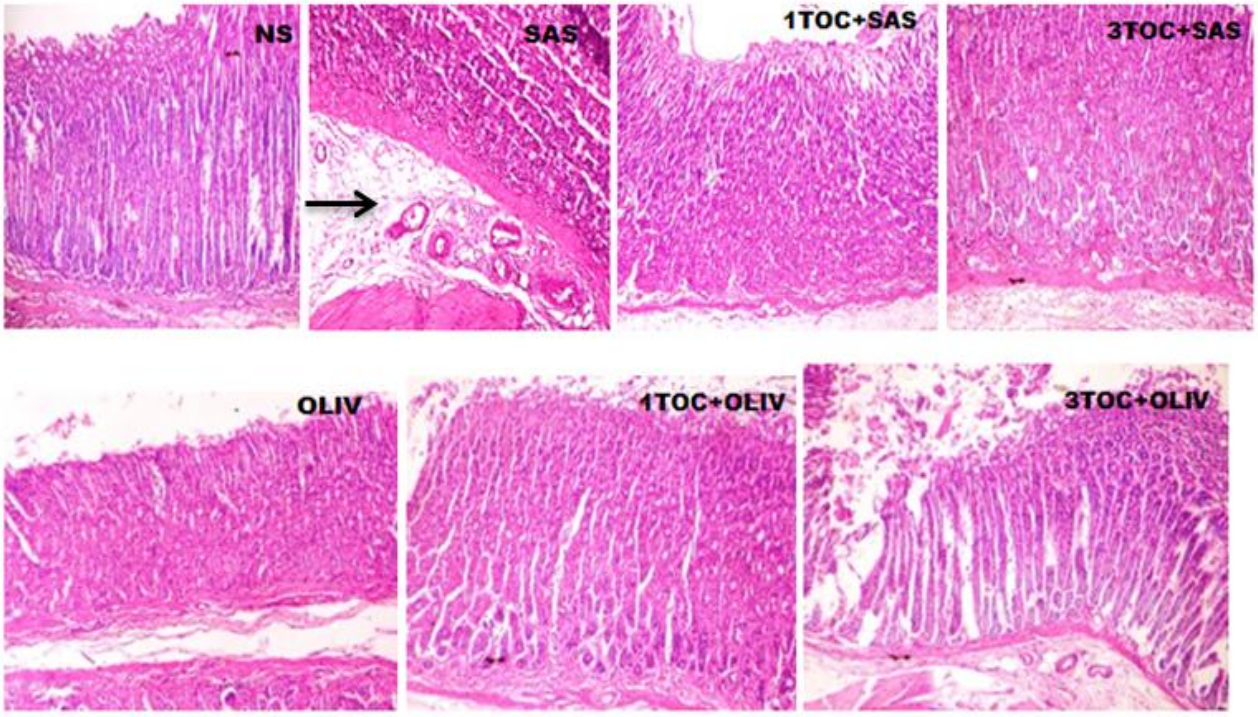

Plate 1:
Histological presentation of normal and sodium arsenite exposed stomach treated with tocopherol. (X100)
NS=Normal mucosa, submucosa and muscularis. The surface epithelial is well preserved. SAS= Mild edema (black arrow) and infiltration by acute inflammatory cells. 1TOC+SAS= Mild inflammation of the mucosa and submucosa. **3TOC+SAS=** Normal mucosa, submucosa and muscularis. The surface epithelial is well preserved. No significant lesion. **OLIV=** Normal mucosa, submucosa and muscularis. The surface epithelial is well preserved. **1TOC+OLIV=**Normal mucosa, submucosa and muscularis. **3TOC+OLIV=**Normal mucosa, submucosa and muscularis.

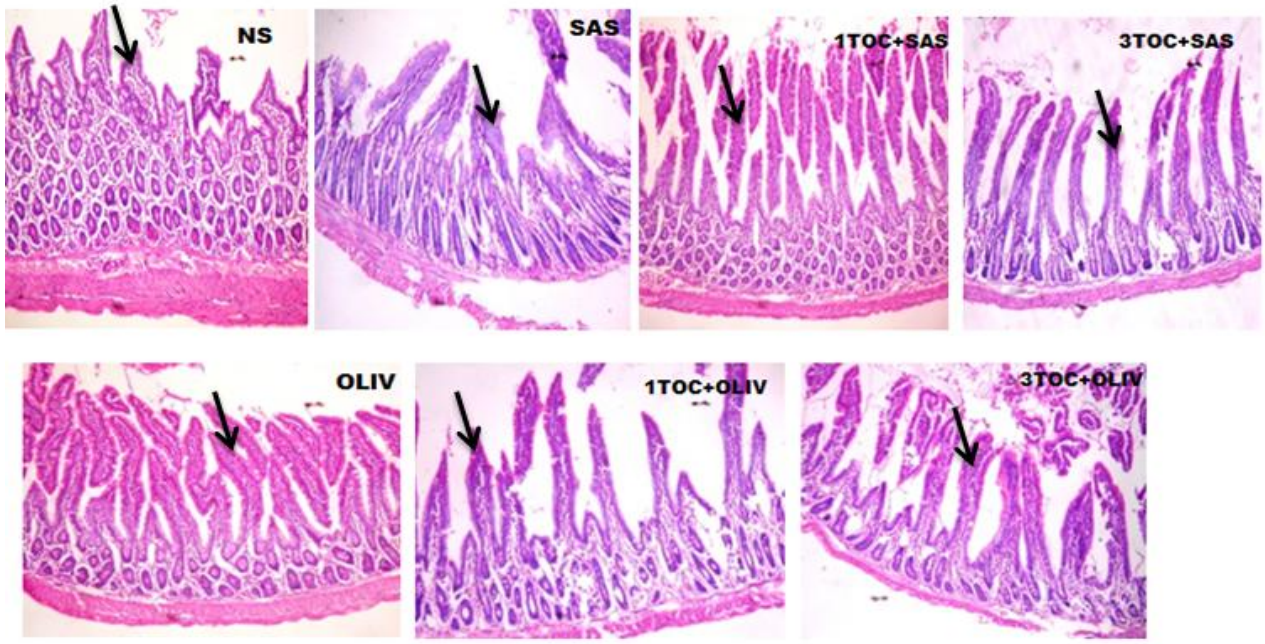

Plate 2:
Histological presentation of normal and sodium arsenite exposed ileum treated with tocopherol. (X100)
NS= Normal villi structure, normal mucosa, submucosa, and muscularis, and normal crypt-villous architecture (black arrow) is well preserved. SAS= Focal area of loss of intestinal glands and gross distortion of the villous-crypt structure. 1TOC+SAS= Normal villi structure, normal mucosa, submucosa and muscularis, and the normal crypt-villous architecture. 3TOC+SAS= Normal villi structure, normal mucosa, submucosa, and muscularis; normal crypt-villous architecture. OLIV= Normal villi structure, normal mucosa, submucosa, and muscularis with normal crypt-villous architecture. 1TOC+OLIV=Normal villi structure, normal mucosa, submucosa, and muscularis, and normal crypt-villous architecture is well preserved. 3TOC+OLIV= Normal villi structure, normal mucosa, submucosa, and muscularis and normal crypt-villous architecture are well preserved.

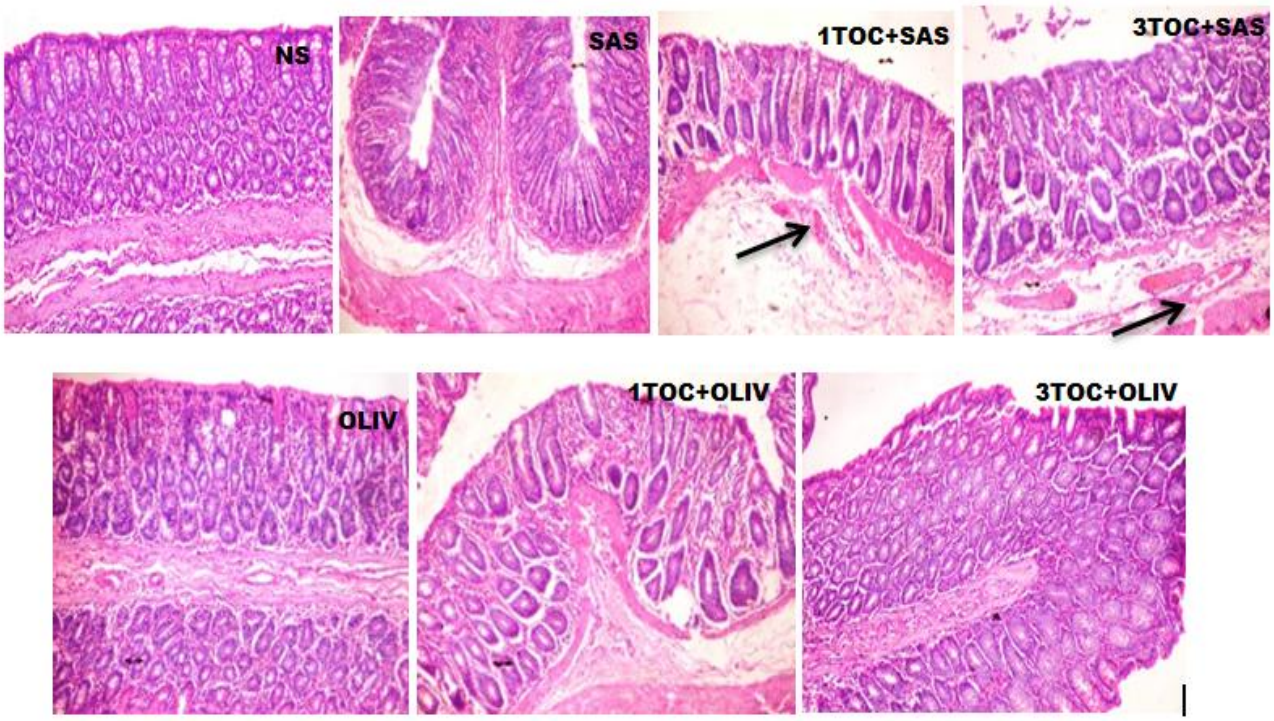

Plate 3:
Histological presentation of normal and sodium arsenite exposed colon treated with tocopherol
NS= normal mucosa, submucosa and muscularis. No significant lesion was seen**.** SAS= Focal area of loss of tubular glands and replacement by chronic inflammatory cells and fibroblasts 1TOC+SAS= mild vascular congestion, 3TOC+SAS= mild vascular congestion (black arrow) and inflammation of the submucosa. OLIV=Normal mucosa, submucosa and muscularis. 1TOC+OLIV= normal mucosa, submucosa and muscularis. 3TOC+OLIV= normal mucosa, submucosa and muscularis.

Table 2:
Effects of graded doses of tocopherol on sodium arsenite-induced toxicity in the rat's ileum: Villus Height, Crypt Depth, and Inflammatory cell infiltration

	Villus Height (HD, µm)	Crypt Depth (CD, µm)	VH/CD	Inflammatory Cell Infiltration score
NS	120±2.5	51±1.8	2.35±0.05	0.4±0.2
SAS	55±1.0*	52±2.0	1.05±0.01*	2.4±0.2*
1TOC+SAS	98±2.0#	55±2.5	1.78±0.02#	1.0±0.3#
3TOC+SAS	100±1.4#	50±1.5	2.00±0.01#	0.8±0.2#
OLIV	110±3.0	51±3.5	2.15±0.01	0.8±0.2
1TOC+OLIV	125±5.0	54±1.0	2.31±0.02	0.6±0.2
3TOC+OLIV	120±1.0	50±2.0	2.40±0.01	0.6±0.2

The value represents means value and standard error, n=5. A significant difference is shown as *p<0.05 when compared with the control (NS), and #p<0.05 when compared with the sodium-arsenite treated (SAS) only.

Table 3:
Effects of graded doses of tocopherol on sodium arsenite-induced toxicity in the rat's colon: Goblet cell count, Crypt Depth, and Inflammatory cell infiltration

	Goblet cell count (Cells/field)	Crypt Depth (µm)	Inflammatory Cell Infiltration score
NS	11.4±0.4	85±1.5	0.6±0.2
SAS	5.8±0.3*	140±2.0*	2.4±0.2*
1TOC+SAS	8.8±0.3#	100±1.7#	1.0±0.3#
3TOC+SAS	9.6±0.4#	95±1.5#	0.6±0.2#
OLIV	10.4±0.3	82±1.0	0.6±0.2
1TOC+OLIV	11.0±1.0	87±2.2	0.6±0.2
3TOC+OLIV	10.8±0.3	80±2.5	0.6±0.2

*The value represents means value and standard error, n=5. A significant difference is shown as *p<0.05 when compared with the control (NS), and #p<0.05 when compared with the sodium-arsenite treated (SAS) only*

Histology: Upon histopathological examination of the gastrointestinal cytoarchitectural integrity, clear alterations and distortions were observed in the epithelium of the sodium arsenite alone group. Sodium arsenite provokes marked inflammatory cellular infiltration in the stomach (Plate 1), focal loss of glands (Plate 2 and 3), hyperplasia of crypts (Plate 3), atrophic villi, and hypertrophy of Peyer's patches in the intestines (Plate 2). All these were mitigated in the tocopherol treated group. With histomorphometry, it was observed that tocopherol annulled sodium arsenite-induced increase in parietal cell mass and decrease in mucous cell density in the stomach (Table 1), decrease in villus height and villus height/crypt depth ratio in the ileum (Table 2), and decrease in goblets cells and increase in crypt depth in the colon (Table 3).

DISCUSSION

In the present study, we presented findings of sodium arsenite toxicity on the gastrointestinal tract, and subsequent improvement by tocopherol intervention. Sodium arsenite is an inorganic compound whose exposure causes damage to the gastrointestinal tract with clinical manifestations such as nausea, vomiting, and diarrhea (Jomova *et al.*, 2011). Previous workers have also reported cytoarchitectural alterations and lesions in the stomach and intestinal epithelium in laboratory animals treated with sodium arsenite (Hemalatha *et al*., 2013).

Reactive Oxygen Species (ROS) plays an important role in lipid peroxidation implicated in the pathogenesis of many diseases. In this study, Arsenite (AS) increased the level of lipid peroxidation (MDA) and Myeloperoxidase activity (MPO) which are indicators of oxidative stress. The increased lipid peroxidation results in oxidative stress which occurs when the dynamic balance between pro-oxidant and antioxidant mechanisms is impaired (Flora *et al.*, 2008). This underscores the fact that AS exerts its effects through the production of free radicals that cause cellular damage as previous findings have shown increased lipid peroxidation even at low doses (Petrick *et al*., 2000). Nevertheless, tocopherol showed a distinct reduction in MDA contents and MPO activity in the stomach, ileum, and colon of the rats, though not clearly in a dose-dependent fashion. The lessening effects of tocopherol might easily be linked to its antioxidant property.

Upon histological examination, a clear correlation was established between increased MPO activity and inflammatory cell infiltration in the GI segments which is a common phenomenon with arsenic toxicity and evidenced in this study. The activated inflammatory cell infiltration observed in the sodium arsenite-treated stomach, ileum, and colon was mitigated by tocopherol. This suggests that the anti-inflammatory effect of tocopherol seen in this study is via lowering the activated and infiltrated inflammatory cells in the tissues and associated MDA and MPO. Several lines of evidence have demonstrated how infiltrated neutrophils and inflammatory cells release reactive oxygen species upon which altered MDA content and MPO activity is predicated (Ajeigbe *et al*., 2014). In the stomach, parietal cells increased while the mucous cells decreased in the arsenite group which was upturned with tocopherol. Parietal cells secrete hydrochloric acid hugely needed for protein digestion, and there exists a direct relationship between parietal cell count and the acid output. Hence, this lends credence to the findings of Adebayo-Gege *et al.*, (2018), that reported parietal cell hyperplasia and elaborated acid secretion in sodium arsenite-treated stomachs. Focal loss of glands, hyperplasia of crypts, atrophic villi, and hypertrophy of Peyer's patches in the intestines of the arsenite treated were all lessened with tocopherol treatment. Khan *et al*., (2013) had equally reported crypt hyperplasia and sloughing of villi in broiler chicks exposed to sodium arsenite. Alteration in the colonic crypt architecture and depletion of goblet cells are other histological ways of grading inflammation in the gastrointestinal system (Kim *et al*., 2012). Interestingly, tocopherol treatment reversed all these.

Further, data from this study revealed enhancement of Superoxide Dismutase and Catalase activities by tocopherol in all the GI segments examined. As the first line of defense against oxidative stress, SOD and CAT are essential in maintaining cellular health and protecting the body from free radicals and other harmful agents. While there was a deficiency of these important enzymes in the gut samples of the group exposed to arsenite, an upgrade in the level of these enzymes was evident in all the groups treated with tocopherol. SOD is known for its ability to convert superoxide radicals into H_2O_2 while CAT breaks H_2O_2 into water molecules. An elevation of superoxide radicals is associated with reduced SOD and CAT (Ighodaro and Akinloye 2018), to a large extent as it is seen in the arsenite-only treated group.

Glutathione serves as a second line of defense in the body and plays an important role in preventing free radicals with its –SH group. Glutathione-related enzymes like GPx, GST function in the detoxification process to regulate cellular homeostasis (Yamanaka *et al.*, 1991). The results from the study showed that there was a significant alteration in the GSH level. For recycling of this enzyme, GPx catalyzes the conversion of GSH into GSSG and regeneration of GSH from GSSG. Hence, activities of GPx and GR are strictly regulated with the changes in the GSH level. In the current study, the decrease observed in both GPx and GST activities is an easily attributable reduction in GSH level in the stomach, ileum, and colon of the arsenite-only treated rats when compared to the control. It can thus be hypothesized that altered glutathione-linked enzymes lead to an oxidative imbalance in all the GI segments examined and precipitated oxidative stress. Meanwhile, tocopherol reversed the depletion of GSH, GST, and GPx.
Alterations of nitric oxide (NO) in the periphery have been detected after arsenic exposure. The nitrergic dysfunction can also be linked to oxidative stress since inadequate levels of tetrahydrobiopterin, one of the cofactors for NO synthesis are thought to contribute to superoxide formation by nitric oxide synthase (NOS) (Kamada *et al*., 2005). In this study, the activity of NO decreased in the arsenite group, which also presented the characteristic effects of lipid peroxidation and increase of ROS production associated with arsenic exposure.

In conclusion, tocopherol is a great dietary supplement that has the potential to mitigate arsenic toxicity on the gastrointestinal system via its anti-oxidative properties.

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