

Gastrointestinal protective efficacy of Kolaviron (a bi-flavonoid from *Garcinia kola*) following a single administration of sodium arsenite in rats: Biochemical and histopathological studies

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ABSTRACT

Background: Arsenic intoxication is known to produce symptoms including diarrhea and vomiting, which are indications of gastrointestinal dysfunction. **Objective:** We investigated whether Kolaviron (KV) administration protected against sodium arsenite (NaAsO₂)-induced damage to gastric and intestinal epithelium in rats. **Materials and Methods:** Control rats (Group I) were given a daily oral dose of corn oil. Rats in other groups were given a single dose of NaAsO₂ (100 mg/kg; intraperitoneal) alone (Group II) or after pretreatment for 7 days with KV at 100 mg/kg (Group III) and 200 mg/kg (Group IV). Rats were sacrificed afterward and portions of the stomach, small intestine and colon were processed for histopathological examination. Hydrogen peroxide, reduced glutathione, malondialdehyde (MDA) concentrations as well as activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione S-transferase (GST) and myeloperoxidase (MPO) were measured in the remaining portions of the different gastrointestinal tract (GIT) segments. **Results:** NaAsO₂ caused significant increases ($P < 0.05$) in MDA levels and MPO activity, with significant reductions ($P < 0.05$) in GST, GPX, CAT and SOD activities in the stomach and intestines. KV significantly reversed the changes ($P < 0.05$) in a largely dose-dependent manner. The different segments had marked inflammatory cellular infiltration, with hyperplasia of the crypts, which occurred to much lesser degrees with KV administration. **Conclusion:** The present findings showed that KV might be a potent product for mitigating NaAsO₂ toxicity in the GIT.

Key words: Chemoprevention, intestines, kolaviron, sodium arsenite, stomach

INTRODUCTION

Arsenic is a toxic metalloid (displaying some properties of both metal and a nonmetal), occurring widely in the air, water and soil, but commonly referred to as a heavy metal in toxicology.^[1,2] Humans are exposed to a wide variety of inorganic forms including arsenates (AsO₄³⁻) or arsenites (AsO₂⁻) in water, soil or food,^[3] especially seafood, rice, mushrooms and poultry products^[4,5] and drinking water contaminated with arsenic.^[6,7]

Oral ingestion of sodium arsenite (NaAsO₂) has been known to produce severe gastrointestinal symptoms, including abdominal pain, diarrhea, and nausea.^[2,8] When arsenic is absorbed, it is distributed to all body tissues, after which it is reduced *in vivo* to trivalent arsenic which becomes methylated in the liver to methylarsonic acid and dimethylarsinic acid.^[9] Inorganic arsenic is rapidly eliminated from the blood via renal elimination, although biliary excretion also does occur, creating the possibility of intestinal exposure to metabolites of arsenic.^[10]

Arsenic has been primarily known to produce toxicity by interaction with enzymes and proteins containing sulfhydryl groups.^[11] This interaction changes the redox status of these proteins and may cause alterations in their

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biological function. Arsenic also induces an increased formation of reactive oxygen (ROS) and nitrogen species, including the superoxide radical and hydroxyl radicals. An increase in lipid peroxidation as a result of the radicals causes production of several bioactive molecules of which malondialdehyde (MDA) and 4-hydroxynonenal are major examples.^[2] Flavonoids are effective hydroxyl radical and peroxy radical scavengers. They can also make complexes with metals and inhibit metal-initiating lipid peroxidation.^[12] Plant polyphenols including flavonoids and carotenoids are usually not as well absorbed as low-molecular-weight antioxidants such as Vitamins C and E. Therefore, the concentrations of dietary flavonoids can be much higher in the lumen of the gastrointestinal tract (GIT) than that in the plasma or other body tissues, making their antioxidant effects more prominent in the gastrointestinal epithelium.^[13] Flavonoids are also known to chelate free iron and copper which could otherwise increase ROS generation.^[14] Several published reports suggest that arsenic causes damage to the body's anti-oxidative system and that this can be prevented by treatment with potential natural antioxidants.^[15]

Kolaviron (KV) [Figure 1] is a fraction of the defatted ethanol extract of *Garcinia kola*, containing Garcinia bioflavonoids GB1, GB2, and Kolaflavanone.^[16] The ability of KV to inhibit hydroxyl and superoxide radicals has been demonstrated.^[17] Structurally, KV possesses many hydroxyl groups on the dual B rings [Figure 1], which confer on it the antioxidant and radical scavenging activities. KV has also been shown to offer protection against gastric ulcers^[18] and ulcerative colitis.^[19] The protective effects of KV against heavy metal intoxication, such as cadmium in the testes and spermatozoa has been demonstrated.^[20]

Arsenic administered orally via drinking water or by oral gavage has been shown to induce oxidative stress and structural impairment in GIT and other tissues.^[21,22] The oral route is also the major means of natural exposure to

arsenic compounds. However, the intraperitoneal route is the most frequently used route of administration in toxicity studies involving arsenic in laboratory animals.^[23]

This protocol will also enable the comparison of parenteral arsenic effects on the GIT with previous reports involving oral exposures in the literature.^[22,24] To our knowledge, no study has been carried out to explore the protective role of KV against arsenic gastrointestinal toxicity. We therefore sought to investigate whether NaAsO₂ administered intraperitoneally induces significant gastrointestinal injury and whether KV could offer protection to gastrointestinal tissues in arsenic-induced toxicity.

MATERIALS AND METHODS

Chemicals

NaAsO₂, Epinephrine, glutathione, 5,5'-dithiobis-2-nitrobenzoic acid, hydrogen peroxide (H₂O₂), 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.

Animal protocol and experimental design

A total of 24 adult male Wistar rats weighing 100–120 g were obtained from the Experimental Animal Unit of Faculty of Veterinary Medicine, University of Ibadan, Nigeria. The animals were kept in plastic cages in a well-ventilated animal house under controlled light cycle (12 h light/12 h dark) and fed with commercial rat chow and water *ad libitum*. All of the animals received humane care according to the criteria outlined in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health. The ethics regulations were followed in accordance with national and institutional guidelines for the protection of the animals' welfare during experiments.^[25] They were randomly divided into four groups of six animals each as follows:

- Group I: Control rats receiving corn oil for 8 days
- Group II: Rats treated with NaAsO₂ at day 8
- Group III: Rats pretreated with KV (100 mg/kg; per oral) for 8 days, followed by single administration of NaAsO₂ (100 mg/kg; intraperitoneal) on the 8th day
- Group IV: Rats pretreated with KV (200 mg/kg; per oral) for 8 days, followed by a single administration of NaAsO₂ (100 mg/kg; intraperitoneal) on the 8th day.

Rats were sacrificed 24 h after the last administration by cervical dislocation, followed by removal of the gastrointestinal segments, stomach, small intestine and colon. The stomach was cut open along the greater curvature while the intestines were opened up along their entire lengths. Small portions of the different segments

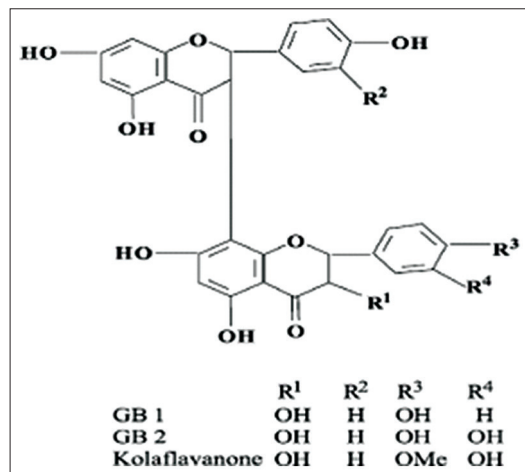


Figure 1: Structure of Kolaviron

were cut for histopathological examination. The remaining portions were rinsed and homogenized using 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% KCl. The homogenate was subjected to cold centrifugation at 4°C at a speed of 10,000x g for 15 min. The supernatant which was taken as the postmitochondrial fraction was used for the estimation of different biochemical parameters.

Biochemical assays

Protein concentration was determined by the Biuret method of Gornal *et al.*^[26] H₂O₂ generation was assessed by the method of Wolff.^[27] MDA concentration as an index of lipid peroxidation was quantified according to the method described by Farombi *et al.*^[28] Reduced glutathione (GSH) concentration was determined using the method of Jollow *et al.*^[29] Catalase (CAT) activity using H₂O₂ as substrate was measured by the method of Claiborne.^[30] Superoxide dismutase (SOD) assay was carried out by the method of Misra and Fridovich^[31] with slight modification in our laboratory.^[32] Glutathione peroxidase (GPX) activity was measured by the method of Rotruck *et al.*^[33] Glutathione S-transferase (GST) was estimated by the method of Habig *et al.*^[34] using 1-chloro-2,4-dinitrobenzene as substrate. Myeloperoxidase (MPO) activity was determined by the method of Xia and Zweier.^[35]

Histopathology

To examine the morphological changes associated with NaAsO₂ administration in the mucosa of the GIT, small portions of the stomach, duodenum, ileum and colon were immediately fixed in 10% formal saline buffer, embedded in paraffin wax, and sections of 5–6 mm in thickness were made and thereafter stained with hematoxylin and eosin for histopathological examination according to Drury *et al.*^[36] The sections were examined by light microscopy.

Statistical analysis

All values are expressed as mean ± standard deviation. The test of significance between two groups was estimated by Student's *t*-test using SPSS (student version 7.5; SPSS Inc., Surrey, UK). Values <0.05 were considered statistically significant.

RESULTS

Effects of Kolaviron on oxidative stress parameters and Myeloperoxidase activity in gastrointestinal tissues of sodium arsenite-treated rats

The protective effect of KV on NaAsO₂-induced lipid peroxidation in stomach and intestines is presented in Figure 2. Although NaAsO₂ did not produce significant changes in MDA values when compared to control values, KV pretreatment, followed by the single dose of NaAsO₂ produced significant reduction ($P < 0.05$) in

lipid peroxidation, compared to both normal control and NaAsO₂ control. This was indicated by reduced MDA level which was more obvious in the stomach and colon. The reduction in MDA was observed to be dose-dependent.

As shown in Figure 3, MPO activity was found to be significantly increased ($P < 0.05$) in all the segments of the GIT (stomach, small intestine, and colon), compared to normal controls. However, KV pretreatment at the two doses tested produced a significant reduction ($P < 0.05$) in MPO activity when compared with NaAsO₂ control. The higher dose of KV (200 mg/kg) produced a significant reduction in MPO activity even below that of normal control.

Hydrogen peroxide concentration was not significantly altered in all the different groups [Table 1]. KV at 200 mg/kg produced a significant reduction ($P < 0.05$) in H₂O₂ in the colon. A reduction in H₂O₂ levels was only observed in the colon at the higher dose of KV.

Effects of Kolaviron on antioxidant systems in gastrointestinal tissues of sodium arsenite-treated rats

Glutathione S-transferase activity was significantly reduced ($P < 0.05$) in NaAsO₂-treated rats in the stomach and intestine, whereas, the activity of GST increased significantly ($P < 0.05$) in animals pretreated with KV in a dose-dependent manner [Figure 4]. CAT activity was also found to be significantly reduced in the small intestine and colon, but not in the stomach upon NaAsO₂ exposure, compared to normal controls [Figure 5]. Again, KV pretreatment caused significant increases ($P < 0.05$) in CAT activity in all the GIT segments compared to rats treated with NaAsO₂ only.

Neither NaAsO₂ administered alone nor following KV pretreatment caused any significant alterations in GSH concentration [Table 2] in the different GIT segments. NaAsO₂-treated animals showed a statistically significant reduction ($P < 0.05$) in GPX activity when compared with control animals [Table 3]. In a similar fashion to GST and CAT activities, KV produced a significant increase ($P < 0.05$) in GPX activity in animals treated with NaAsO₂ following KV pretreatment. SOD activity in the stomach and small intestine was significantly enhanced ($P < 0.05$) by KV pretreatment when compared with animals treated with NaAsO₂ only [Table 4].

Microscopy

Microscopic examination of the gastrointestinal epithelium revealed considerable pathology associated with NaAsO₂ exposure, much of which were reduced with KV pretreatment. Lesions in the stomach epithelium were predominantly infiltration of the mucosa and submucosa by inflammatory

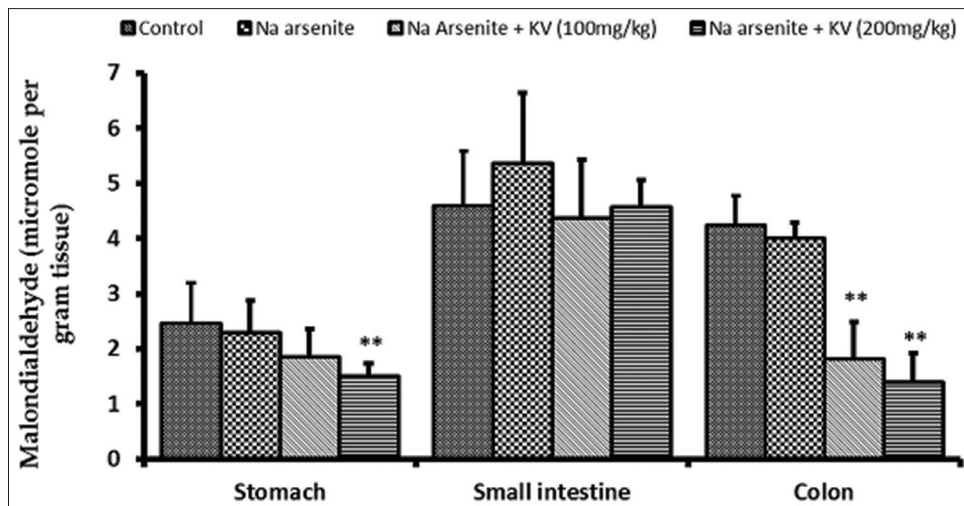


Figure 2: Effect of Kolaviron (KV) on malondialdehyde levels in the stomach and intestines of sodium arsenite-treated Wistar rats. Values are expressed as mean ± standard deviation of six rats **P* < 0.05 by Student's *t*-test when the values of group II (Na arsenite) are compared with those of group I (control) ***P* < 0.05 by Student's *t*-test when the values of groups III (Na arsenite + 100 mg/kg KV) and IV (Na arsenite + 200 mg/kg KV) are compared with those of group II (Na arsenite)

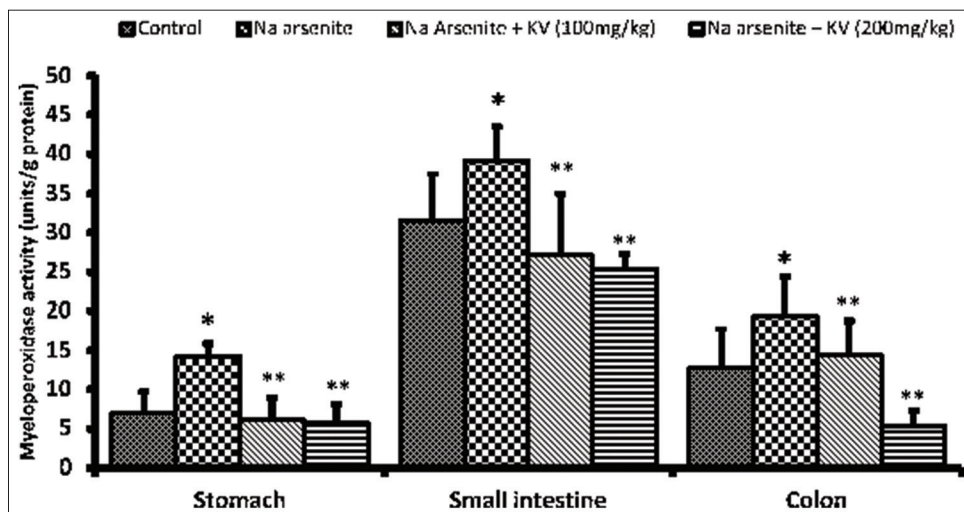


Figure 3: Effect of Kolaviron (KV) on myeloperoxidase activity in the stomach and intestines of Sodium arsenite-treated Wistar rats. Values are expressed as mean ± standard deviation of six rats **P* < 0.05 by Student's *t*-test when the values of group II (Na arsenite) are compared with those of group I (control) ***P* < 0.05 by Student's *t*-test when the values of groups III (Na arsenite + 100 mg/kg KV) and IV (Na arsenite + 200 mg/kg KV) are compared with those of group II (Na arsenite)

cells [Figure 6]. KV-treated rats, however, exhibited significantly lesser degrees of inflammatory cell infiltration. In the ileum and duodenum [Figures 7-9, respectively], NaAsO₂ treatment-induced pronounced hyperplasia of the crypts and cells lining the glands at the base of the mucosa. Some of the nuclei appeared vesicular with increased nucleocytoplasmic ratio. In these GIT segments, there was also marked inflammatory cell infiltration as seen in the stomach of NaAsO₂ treated rats. Some focal areas of erosion and ulceration were observed in the duodenum and ileum (not shown). However, with KV treatment, these lesions were considerable reduced.

Table 1: Effect of KV on H₂O₂ generation (micromole per minute per mg protein) in the stomach and intestines of NaAsO₂-treated Wistar rats

Treatment groups	Gastrointestinal tract segment		
	Stomach	Small intestine	Colon
Control	11.00±0.53	10.19±0.97	11.05±0.33
NaAsO ₂	11.17±0.72	10.45±1.19	11.5±0.43
NaAsO ₂ +KV (100 mg/kg)	11.80±0.45	11.13±0.43	11.10±0.38
NaAsO ₂ +KV (200 mg/kg)	11.17±0.75	10.81±1.05	10.31±0.52**

Values are expressed as mean±SD of six rats. ***P* < 0.05 by Student's *t*-test when the values of groups III (Na arsenite+100 mg/kg KV) and IV (Na arsenite+200 mg/kg KV) are compared with those of group II (Na arsenite). KV=Kolaviron; H₂O₂=Hydrogen peroxide; NaAsO₂=Sodium arsenite

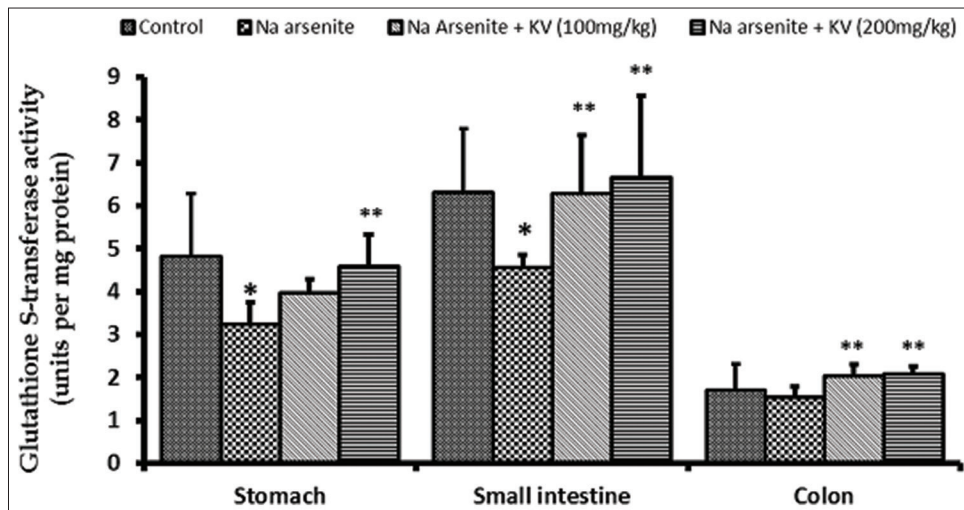


Figure 4: Effect of Kolaviron (KV) on Glutathione S-transferase activity in the stomach and intestines of sodium arsenite-treated Wistar rats. Values are expressed as mean \pm standard deviation of six rats * $P < 0.05$ by Student's *t*-test when the values of group II (Na arsenite) are compared with those of group I (control) ** $P < 0.05$ by Student's *t*-test when the values of groups III (Na arsenite + 100 mg/kg KV) and IV (Na arsenite + 200 mg/kg KV) are compared with those of group II (Na arsenite)

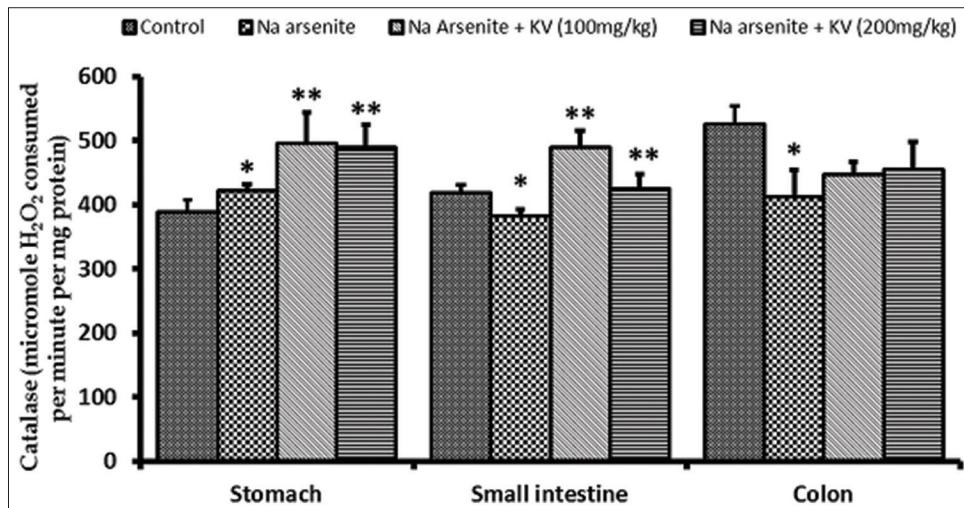


Figure 5: Effect of Kolaviron (KV) on Catalase activity in the stomach and intestines of sodium arsenite-treated Wistar rats. Values are expressed as mean \pm standard deviation of six rats * $P < 0.05$ by Student's *t*-test when the values of group II (Na arsenite) are compared with those of group I (control) ** $P < 0.05$ by Student's *t*-test when the values of groups III (Na arsenite + 100 mg/kg KV) and IV (Na arsenite + 200 mg/kg KV) are compared with those of group II (Na arsenite)

DISCUSSION

This study was designed to investigate the nature of gastrointestinal involvement in NaAsO₂-induced toxicity, when the latter is administered intraperitoneally. We also examined the possibility of protection by KV on alterations produced. NaAsO₂ exposure is known to produce clinical signs of gastrointestinal dysfunction including nausea, vomiting, and diarrhea while hemorrhagic gastrointestinal lesions have been reported in some animal studies.^[2,37,38] Earlier studies in rats have shown that orally administered NaAsO₂ in rats produced histological alterations in the gastric and intestinal epithelium with vacuolation and rupture and degeneration of villi and the gastric mucosa.^[24]

In this study, pretreatment with KV and eventual NaAsO₂ administration did not cause any significant changes in the weights of the stomach, small intestine and colon in all the groups (values not presented).

Arsenic exposure has been associated with the production of reactive oxygen intermediates causing lipid peroxidation and cellular damage.^[22] Our results indicated that there were no significant changes in H₂O₂ generation and MDA concentration in rats treated with NaAsO₂ alone compared to the control in all the segments of the GIT. MDA measured as an index of lipid peroxidation represents an important end product in the process. This finding from the present study may suggest that the single administration

Table 2: Effect of KV on reduced GSH levels (micromole per gram tissue) in the stomach and intestines of NaAsO₂-treated Wistar rats

Treatment groups	Gastrointestinal tract segment		
	Stomach	Small intestine	Colon
Control	49.29±3.51	49.29±2.27	45.04±0.71
NaAsO ₂	47.54±1.11	53.33±4.34	44.42±0.61
NaAsO ₂ +KV (100 mg/kg)	49.33±5.48	48.54±2.74	43.04±0.49
NaAsO ₂ +KV (200 mg/kg)	49.88±4.60	55.83±5.08	42.92±0.72

Values are expressed as mean±SD of six rats. KV=Kolaviron; GSH=Glutathione; NaAsO₂=Sodium arsenite

Table 3: Effect of KV on GPx activity (units per mg protein) in the stomach and intestines of NaAsO₂-treated Wistar rats

Treatment groups	Gastrointestinal tract segment		
	Stomach	Small intestine	Colon
Control	377.58±38.01	295.53±14.21	409.08±19.53
NaAsO ₂	394.28±21.43*	288.23±3.66*	360.69±28.04*
NaAsO ₂ +KV (100 mg/kg)	412.97±11.97**	313.95±22.67**	445.24±20.44**
NaAsO ₂ +KV (200 mg/kg)	453.94±34.99**	389.84±21.10**	402.61±15.30**

Values are expressed as mean±SD of six rats. *P<0.05 by Student's t-test when the values of group II (Na arsenite) are compared with those of group I (control). **P<0.05 by Student's t-test when the values of groups III (Na arsenite+100 mg/kg KV) and IV (Na arsenite+200 mg/kg KV) are compared with those of group II (Na arsenite). GPx=Glutathione peroxidase; KV=Kolaviron; NaAsO₂=Sodium arsenite

Table 4: Effect of KV on SOD activity (units per mg protein) in the stomach and intestines of NaAsO₂-treated Wistar rats

Treatment groups	Gastrointestinal tract segment		
	Stomach	Small intestine	Colon
Control	4.36±0.57	3.98±0.13	0.79±0.07
NaAsO ₂	4.73±0.96	3.65±0.23*	0.81±0.03
NaAsO ₂ +KV (100 mg/kg)	6.32±0.52**	4.91±0.86**	0.79±0.09
NaAsO ₂ +KV (200 mg/kg)	7.65±0.74**	5.27±0.33**	0.62±0.09

Values are expressed as mean±SD of six rats. *P<0.05 by Student's t-test when the values of group II (Na arsenite) are compared with those of the group I (control). **P<0.05 by Student's t-test when the values of groups III (Na arsenite+100 mg/kg KV) and IV (Na arsenite+200 mg/kg KV) are compared with those of group II (Na arsenite). SOD=Superoxide dismutase; KV=Kolaviron; NaAsO₂=Sodium arsenite

of NaAsO₂ through the intraperitoneal route may not have caused significant alterations in these parameters to cause sufficient distribution of metabolites of NaAsO₂ to the GIT.

However, it was observed that there was a significant increase in MPO activity in all the GIT segments with NaAsO₂ exposure compared to the controls. MPO is the most abundant pro-inflammatory enzyme stored in the azurophilic granules of neutrophils. It catalyzes the

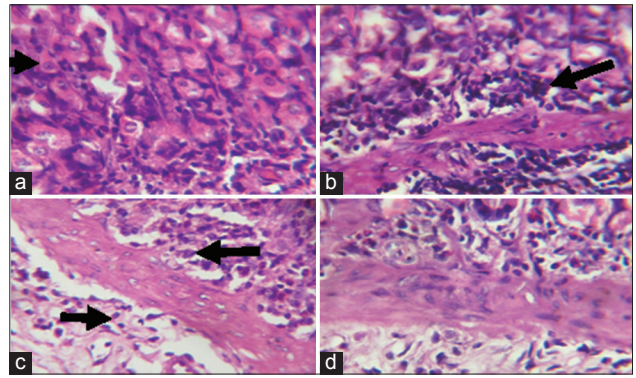


Figure 6: Photomicrographs showing the gastric mucosa of (a) control rats with predominantly normal parietal cells (black arrow) with only few inflammatory cells; (b) Sodium arsenite-treated rats, showing marked infiltration of the mucosa and submucosa with inflammatory cells; (c) rats pretreated with Kolaviron (KV) 100 mg/kg and (d) rats pretreated with KV 200 mg/kg, both showing only mild infiltration at the base of the mucosa

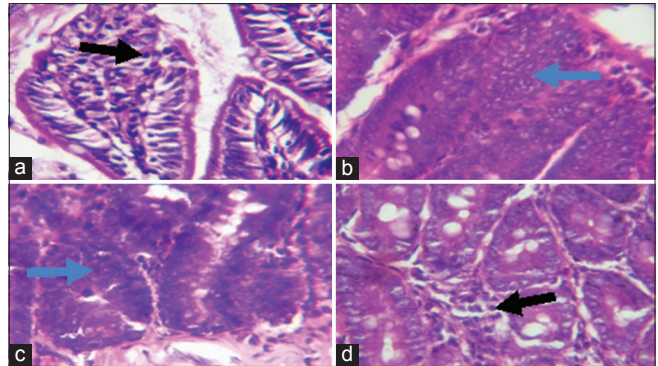


Figure 7: Photomicrographs showing the duodenum of (a) control rats with normal villi (black arrow) (b) sodium arsenite-treated rats, disseminated inflammatory cell infiltration as well as marked hyperplasia of the cells lining the glands (blue arrow) (c) rats pretreated with Kolaviron (KV) 100 mg/kg, also showing hyperplasia of the glands and (d) rats pretreated with KV 200 mg/kg, showing only mild inflammatory cell infiltration

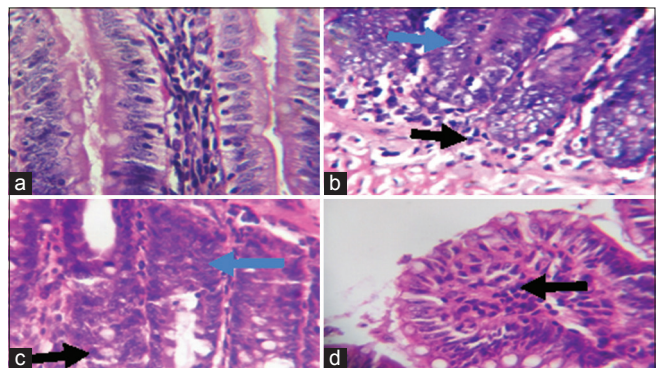


Figure 8: Photomicrographs showing the ileum of (a) control rats with well-arranged villi and few inflammatory cells; (b) sodium arsenite-treated rats, showing hyperplasia of cells lining the glands (blue arrow), some nuclei appearing vesicular as well as increased nucleocytoplasmic ratio and inflammatory cell infiltration; (c) rats pretreated with Kolaviron (KV) 100 mg/kg showing hyperplasia of the crypts and mild inflammatory cell infiltration, and (d) rats pretreated with KV 200 mg/kg, showing only mild infiltration at the base of the mucosa

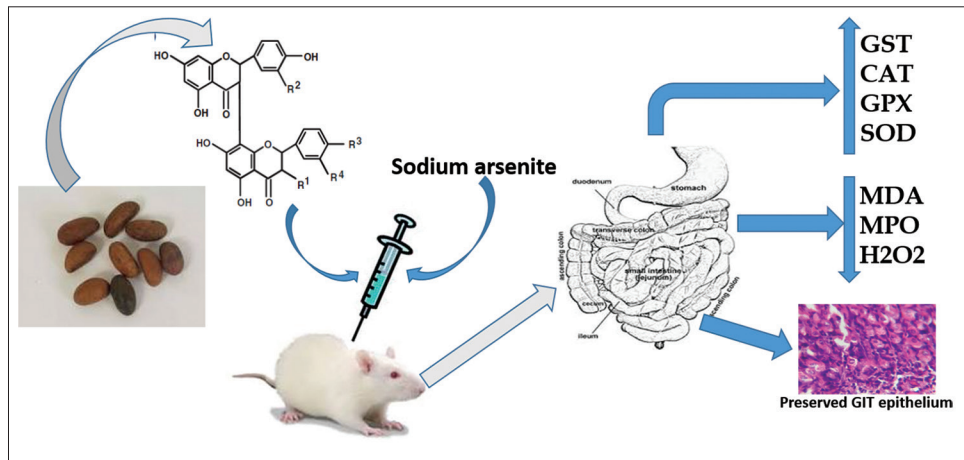


Figure 9: Kolaviron protects against sodium arsenite-induced gastrointestinal toxicity

formation of hypochlorous acid from H_2O_2 . Extracellular MPO activity gives an estimate of the oxidative stress in inflammatory diseases while intracellular MPO activity correlates well with tissue neutrophil content.^[39]

Kolaviron appears to be a very potent gastrointestinal protective agent from the results of our study. Pretreatment of rats before $NaAsO_2$ exposure caused significant reductions in MDA content and MPO activity in a largely dose-dependent manner compared to the rats given $NaAsO_2$ alone. Its anti-inflammatory action on MPO activity appeared to be much more pronounced than its effect on MDA, and this was obtained in all of the GI segments. Results from this study support previous studies demonstrating the antioxidant and anti-inflammatory potentials of KV against GI mucosal injury.^[18,19]

Increased MPO activity in this study correlates with the extent of inflammatory cell infiltration observed with histological examination in the different GI segments studied. One consistent feature of $NaAsO_2$ exposure in the GIT was inflammatory cell infiltration of the mucosa and submucosa of the stomach, ileum, and colon. This points to a general inflammatory state induced by $NaAsO_2$ exposure. In addition, there were areas of marked hyperplasia of the crypts and cells lining the glands at the base of the mucosa. These findings represent a generalized response to the inflammatory state of the tissues.^[40] Crypt hyperplasia is caused by an increase in mitotic activity of crypt epithelial cells and is generally thought to be a compensatory response to toxicity in the gastrointestinal epithelium.^[41] Sloughing of the villi and inflammatory cell infiltration were observed in broiler chicks fed 150 ppm of sodium arsenate.^[42] It was, however, observed that rats pretreated with KV exhibited these pathologies only to mild degrees, with reductions in inflammatory cell infiltration and hyperplasia of crypts. It

is noteworthy that KV at the higher dose exhibited greater protection against histopathological alterations.

Further protection of the GI tissues by KV obtained in this study included an enhancement of the activities of the antioxidant enzymes including SOD, CAT, GST, and GPX. SOD and CAT represent two basic components of the first line of defense against oxidative stress. While SOD converts superoxide radicals to H_2O_2 , CAT converts H_2O_2 into water molecules.^[43]

The reduction in the activities of SOD and CAT obtained in this study indicates increased production of superoxide radicals.^[44] The GSH-related enzymes GST and GPX cause reduction in hydroperoxides within membranes in the presence of GSH. GSH itself is an important tri-peptide component of the cellular defense mechanism against peroxidative attacks.^[43] Both GST and GPX activities were reduced with $NaAsO_2$ exposure in this study. This could ordinarily be attributed to an inhibition of GSH, which is required as a co-factor for both enzymes. However, since levels of GSH measured in this study did not show significant alterations, the inhibition of GST and GPX could be due to other mechanisms. The presence of selenium in GPx, for example, could lead to an inhibition of its activity through the formation of arsenic-selenium complexes.^[45] It is recognized that the primary biochemical mechanism of arsenic toxicity is binding of the metal to the sulfhydryl groups of proteins, resulting in the inhibition of numerous cellular enzyme systems.^[46] The resulting cellular destruction of damaged thiol proteins may enhance production of toxic oxygen radicals.^[47]

CONCLUSION

The present study gives evidence to alterations caused by $NaAsO_2$ manifested mainly by an inflammatory

response in virtually all the segments of the GIT, even when the compound was administered intraperitoneally. KV, however, showed very promising attributes as a protective agent against the NaAsO₂-induced alterations. The anti-inflammatory properties of KV in this study particularly warrant more comprehensive studies.

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