Volume 46
Issue 2 *2022*

Article 6

1-1-2022

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Original Article

Thai Journal of Pharmaceutical Sciences

# Virgin coconut oil ameliorates arsenic hepatorenal toxicity and NO-mediated inflammation through suppression of oxidative stress in rats

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**Received:** September 10, 2021
**Accepted:** November 16, 2021
**Published:** March 23, 2022

## ABSTRACT

**Background:** Compelling evidence has implicated oxidative stress as a leading mechanism for arsenic (As)-induced pathologies. We evaluated antioxidant effect of virgin coconut oil (VCO) against arsenic-induced oxidative stress-mediated hepatorenal damage in rats. **Methods:** Rats were randomly divided into three groups: Control group, as group, and VCO + As group. VCO was orally administered before and along with arsenic (10 mg/kg body weight, orally) for 21 days. Subsequently, serum liver enzymes, albumin (ALB), creatinine, urea, antioxidant enzymes, malondialdehyde, and nitric oxide (NO) level were evaluated. The histology of the tissues was analyzed with standard procedures. **Results:** Arsenic caused marked increases in serum alanine aminotransferase and aspartate aminotransferase activities, along with urea and creatinine levels, whereas ALB level decreased markedly compared to control. Arsenic significantly reduced superoxide dismutase, catalase, and glutathione peroxidase activities, while NO and malondialdehyde levels prominently increased compared to control. In contrast, VCO supplementation before and along with As exposure significantly improved all biochemical parameters and restored the antioxidant defenses comparable to normal control in consistent with improvements in liver and kidney histology. **Conclusion:** Our findings suggest that VCO could protect against As-induced oxidative stress-mediated hepatorenal damage through reducing inflammatory NO levels and increasing antioxidant defense apparatus in rats.

**Keywords:** Antioxidants, arsenic, hepatotoxicity, oxidative stress, toxicity, virgin coconut oil

## INTRODUCTION

Arsenic (As) is a metalloid carcinogen of increasing worldwide health concerns.[1] A number of millions of people are exposed to As toxicity through food and drinking water, and exposure is well known to be a trigger of systemic toxicity.[2] Epidemiological studies indicate that water sources with low arsenic levels are scarce.[3,4] At present, As is found in rice consumed by more than 50% of the world's population.[5] The literature reports deleterious effects of As associated with chronic disease development, including cancer, diabetes, and lung diseases.[6] Inorganic arsenic, particularly the trivalent form from arsenite, is more toxic to humans than the pentavalent form from arsenate.[7] In animal models, arsenite exposure is implicated to induce damage in cardiac, renal, and hepatic tissues.[8,9]

However, the exact mechanism underlying As toxicity is currently unknown. Although some intracellular alterations are proposed; oxidative stress is implicated in arsenic-induced systemic defects and well supported by several studies.[6] The liver and kidney are critical targets of As. Liver is the site of arsenic metabolism, which, hence, is vulnerable to interact with various arsenic species. Arsenite is second in binding ability to hepatocytes, and its hepatic metabolism under physiological conditions leads to reactive oxygen species (ROS) generation.[10] The first-line antioxidant defense systems have been reported altered in As exposures.[6] Arsenic toxicity depresses activities of antioxidant enzymes and glutathione levels through ROS attack, leading to oxidative stress in hepatorenal tissue.[6,8,9,11] Given the ubiquitous nature of As, human exposure to arsenicals from drinking water and foods may be unavoidable. Therefore, inclusion of protective dietary strategy would be attractive to mitigate arsenic toxicity.

Virgin coconut oil (VCO) is emerging as functional food oil amenable to daily diet with health-promoting properties. Recent evidence demonstrates antioxidant and pharmacological activities of VCO in pre-clinical studies.[12] VCO is extracted from coconut (*Cocos nucifera L*) without industrial or chemical treatment.[13] Studies on VCO contents reveal flavonoids and phenolic acids.[14] Moreover, an increasing number of systematic studies indicate that VCO diet inhibits drug-induced oxidative stress-mediated damage.[12,15,16] At present, the beneficial role of VCO supplementation in As toxicity and/or As-induced oxidative stress-mediated hepatorenal damage is unknown. Therefore, the study sought to explore the potential antioxidant effect of VCO supplementation on arsenic-induced oxidative stress and hepatorenal damage in rats.

## MATERIALS AND METHODS

### Chemicals

Sodium arsenite ($NaAsO_2$) was purchased from BDH Chemicals Ltd., Poole, England. The kits used for biochemical assays were obtained from Randox Laboratory Ltd., UK. Thiobarbituric acid (TBA) was purchased from Hi Media Laboratories Pvt. Ltd., India. The dose of sodium arsenite used in this study was based on the previous reports.[17] All other chemicals were of analytical grade.

### Animals

Adult male Wistar rats (8–10 weeks old, weighing 140–170 g) were procured from a Veterinary Breeding House, Nsukka, Enugu State, Nigeria. The rats were housed at the Department of Chemistry/Biochemistry, Alex Ekwueme Federal University, Ndufu-Alike Ikwo, Ebonyi State, Nigeria under standard conditions (25 ± 2° C, 12 h light and 12 h dark). The animals were supplied pelleted commercial growers mash (Vital Feeds Nigeria Ltd., Jos, Nigeria) and tap water *ad libitum*. The acclimatization period was 2 weeks and was handled in humane manner according to the approved animal experimental procedures given by the NIH Publication (NIH Publication No. 85–23, revised 1996) on Guide for the Care and Use of Laboratory Animals.

### VCO

Fresh coconuts were used to prepare VCO according to the method of Nevin and Rajamohan,[18] as modified by Famurewa *et al.*[12] The edible part of coconuts (*Cocos nucifera L.*) was grind into viscous slurry after which 400 ml of distilled water added and sieved by cheesecloth. The coconut milk obtained was left standing for 48 h. The top layer was gently separated and mildly heated (50° C) to remove water content. Thus, the oil that separated from the top layer was scooped out and filtered into an air-tight container and used for this study.

### Experimental Design

Eighteen rats were divided into three groups ($n$ = 6) following 2 weeks of acclimatization. Arsenic was administered to rats as sodium arsenite ($NaAsO_2$). The design of experimental treatment was as follows:

Group 1 (Normal control): Distilled water for 21 days (5 ml/kg body weight of rat)

Group 2 (Arsenic control): As (10 mg/kg body weight of rat) from day 15 to 21.[17]

Group 3 (VCO + Arsenic): VCO (5 ml/kg body weight of rat) from day 1 to 21 + As (10 mg/kg body weight of rat) from day 15 to 21.[12,17]

After the experimental period (21 consecutive days), the rats were overnight fasted and sacrificed under mild diethyl ether anesthesia. The blood samples were obtained by cardiac puncture into plain sample bottles. After 30 min, samples were subjected to centrifugation at 3000 g for 10 min to obtain serum samples used for biochemical analyses. The tissues for histological analysis were cut out and stored in 10% buffered formalin.

### Biochemical Analyses

Superoxide dismutase (SOD) was analyzed by Marklund and Marklund.[19] Catalase (CAT) activity was determined by Aebi.[20] Glutathione peroxidase (GPx) was determined by Flohe and Gunzler,[21] while lipid peroxidation was estimated by the TBA reactive substances (TBARS) expressed in terms of malondialdehyde (MDA) content according to Ohkawa *et al.*,[22] nitric oxide (NO) was measured by Green *et al.*[23] The liver and kidney function markers were estimated according to instructions indicated in Randox kits.

### Histopathological Examination

Liver and kidney tissues were fixed in 10% formalin for 48 h and dehydrated in ethanol and then embedded in paraffin blocks. The blocks were cut into 5 μm sections using a microtome, fixed on slides followed with hematoxylin and eosin (H and E) staining. The prepared slides were observed under light microscope.

### Statistical Analysis

Results are expressed as arithmetic mean ± SEM. Statistical analysis was conducted using ANOVA followed by Tukey's *post hoc* test (SPSS version 22.0 for windows, Inc., Chicago, IL, USA), and $P < 0.05$ was considered significant.

## RESULTS

### Effect of VCO Supplementation and Arsenic on Body Weight on Rats

Table 1 depicts the effect of VCO supplementation on body weight of arsenic-intoxicated rats. Arsenic exposure failed to exert significant ($P > 0.05$) effect on the weight of rats in this study. Arsenic administration for 1 week (day 15–21) exerted no significant changes ($P > 0.05$) on body weight of rats in comparison to normal rats.

### Effect of VCO Supplementation on Hepatorenal Function Markers in Arsenic-intoxicated Rats

In Table 2, as exerted significant ($P < 0.01$) increases in serum hepatic and renal markers. The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were significantly elevated ($P < 0.01$) in serum compared to control. Albumin (ALB) level was considerably ($P < 0.01$) decreased in serum in comparison to control. Renal function markers, creatinine, and urea were significantly increased ($P < 0.01$) by As compared to normal control. Interestingly, supplementation of VCO before and along with As significantly modulated and reversed the biochemical alterations ($P < 0.01$) in the present study.

### Effect of VCO Supplementation on Oxidative Stress Markers in Arsenic-intoxicated Rats

In Figures 1-5, oral administration of sodium arsenite significantly depressed the serum activities of SOD, CAT, and GPx ($P < 0.05$), whereas the levels of lipid peroxidation marker, MDA, and NO considerably increased ($P < 0.05$) when compared with normal control. However, the VCO supplementation resisted the As-induced alterations and significantly enhanced SOD, CAT, and GPx activities. The MDA level and NO decreased markedly ($P < 0.05$) by VCO comparable to normal control.

**Table 1:** Effect of VCO supplementation on body weight of arsenic-intoxicated rats

| Group | Body weight (g) | | |
|---|---|---|---|
| | Day 1 | Day 15 | Day 22 |
| Control | 159±3.8 | 186±6.9 | 182±6.5 |
| Arsenic | 163±1.7 | 194±7.6 | 193±7.1 |
| VCO+Arsenic | 165±5.6 | 186±8.9 | 187±7.2 |

Values are expressed as mean±SEM; $n$=6; VCO: Virgin coconut oil (5 ml/kg bw)

### Effect of VCO on Liver and Kidney Histology

In Figure 6, arsenic caused a severe degenerative necrosis consistent with infiltration of inflammatory cells. However, VCO oral intake inhibited the toxic effect with reduced necrosis without inflammatory cells. In Figure 7, arsenic-induced inflamed glomeruli and necrotic tubules, which was reversed by VCO administration in rats.

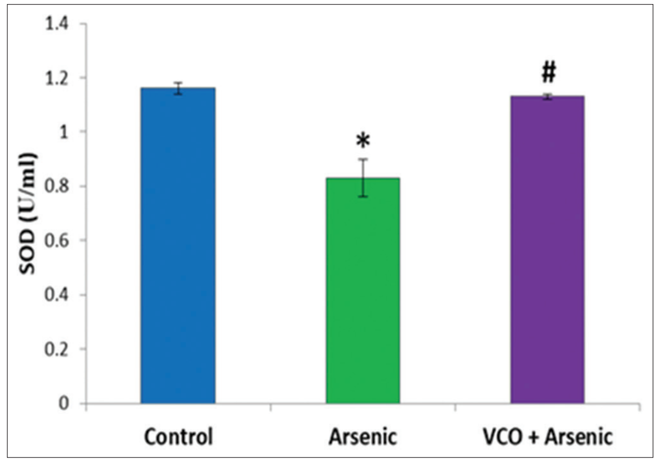


**Figure 1:** Effect of virgin coconut oil on superoxide dismutase activity in arsenic-intoxicated rats. Values are expressed as mean ± SEM; $n = 6$; *significantly different from normal control ($P < 0.01$); #significantly different from arsenic control ($P < 0.01$)

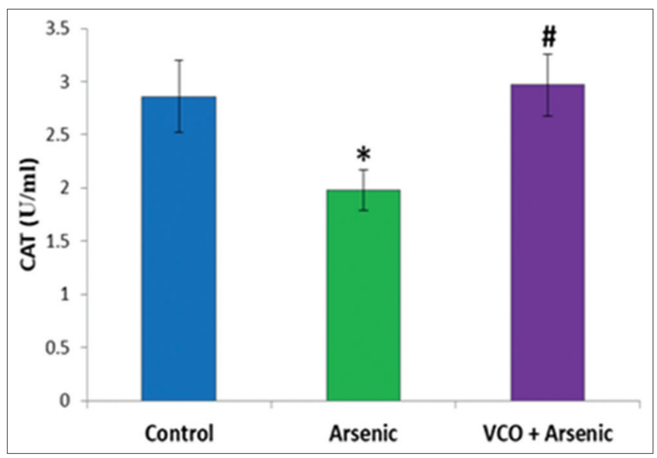


**Figure 2:** Effect of virgin coconut oil on catalase activity in arsenic-intoxicated rats. Values are expressed as mean ± SEM; $n = 6$; *significantly different from normal control ($P < 0.05$); #significantly different from arsenic control ($P < 0.05$)

**Table 2:** Effect of VCO supplementation on body weight of arsenic-intoxicated rats

| Group | ALT (U/L) | AST (U/L) | ALB (g/dl) | Creat (mg/dl) | Urea (mg/dl) |
|---|---|---|---|---|---|
| Control | 6.53±0.61 | 50.56±2.05 | 1.50±0.08 | 1.78±0.02 | 32.62±1.49 |
| Arsenic | 12.02±1.16* | 74.16±3.16* | 0.88±0.13* | 3.19±0.24* | 47.66±1.25* |
| VCO+Arsenic | 5.03±0.31# | 62.55±1.66# | 1.43±0.07# | 1.29±0.02# | 34.95±1.55# |

Values are expressed as mean±SEM; $n$=6; Creat: Creatinine, VCO: Virgin coconut oil; *significantly different from normal control ($P$<0.01); #Significantly different from arsenic control ($P$<0.01)

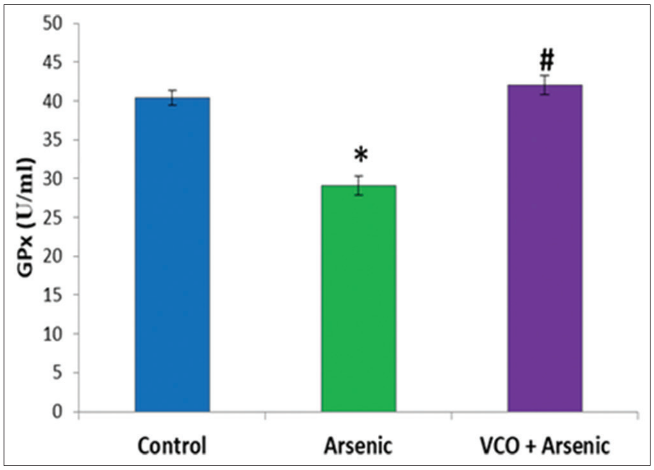


**Figure 3:** Effect of virgin coconut oil on glutathione peroxidase activity (GPx) in arsenic-intoxicated rats. Values are expressed as mean±SEM; $n$=6; *significantly different from normal control ($P < 0.01$); #significantly different from arsenic control ($P < 0.01$)

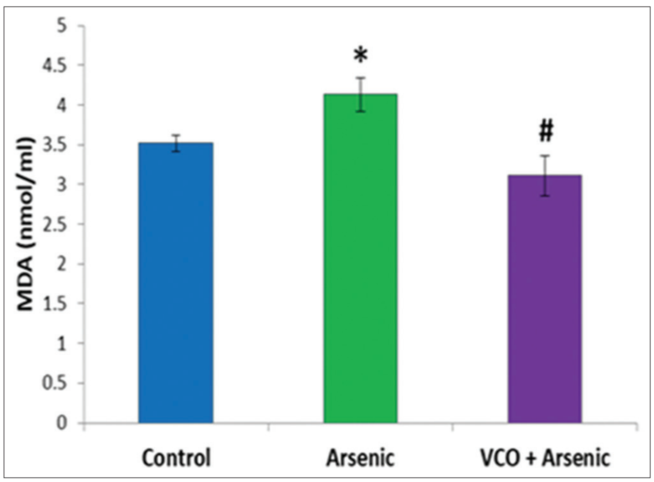


**Figure 4:** Effect of virgin coconut oil on malondialdehyde level in arsenic-intoxicated rats. Values are expressed as mean±SEM; $n$=6; *significantly different from normal control ($P < 0.05$); #significantly different from arsenic control ($P < 0.05$)

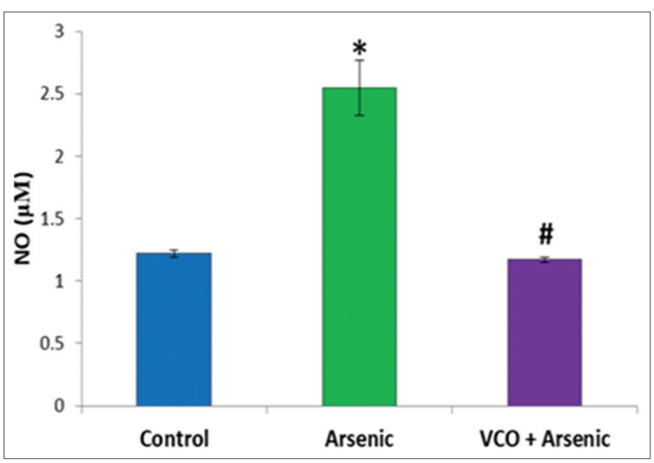


**Figure 5:** Effect of virgin coconut oil on nitric oxide level in arsenic-intoxicated rats. Values are expressed as mean±SEM; $n$=6; *significantly different from normal control ($P < 0.01$); #significantly different from arsenic control ($P < 0.01$)

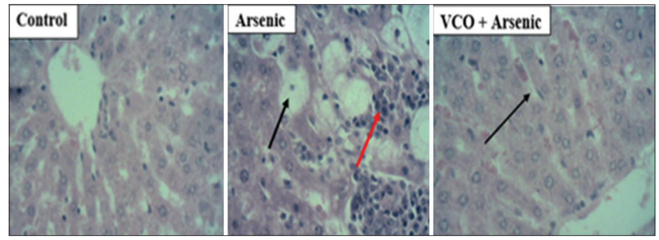


**Figure 6:** Effect of virgin coconut oil (VCO) on hepatic histology in arsenic-treated rats. Photomicrographs of liver sections stained with H and E (400×). Control: Normal architecture with central vein; Arsenic: Severe hepatocellular degeneration and necrosis (black arrow), infiltrated inflammatory cells (red arrow); and VCO + Arsenic: Mild necrosis without inflammatory cells

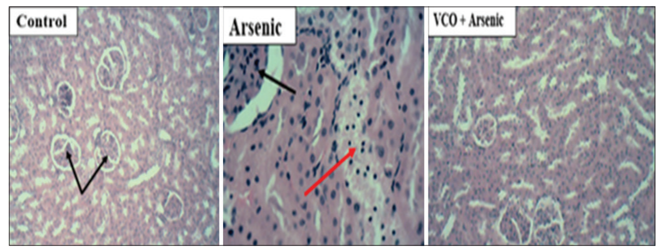


**Figure 7:** Effect of virgin coconut oil (VCO) on renal histology in arsenic-treated rats. Photomicrographs of kidney sections stained with H and E (400×). Control: Normal glomeruli in Bowman's capsule (black arrow); Arsenic: Severely inflamed glomeruli and necrotic tubules (red arrow); and VCO + Arsenic: Near normal glomerulus with epithelial cells

## DISCUSSION

Arsenic is a global pollutant requiring an effective remedy that could combat its toxicities. Its exposure is associated with several pathologies, and oxidative stress is implicated as a leading mechanism underlying its systemic toxicity.[24] Evidence supports the antioxidant and cytoprotective efficacy of natural products to inhibit oxidative mechanisms and preserve biological machinery.[25] Given the increasing reports on the pharmacological activity of VCO,[26] the antioxidant activity of VCO against As-induced oxidative stress-mediated hepatorenal toxicity is explored in this study.

The present study indicates that As-induced hepatic and renal damage through oxidative stress. Transaminases (AST and ALT) are predominantly found in the cytosol and released into the circulatory system during cellular damage. The elevated activities of serum ALT and AST coupled with marked decrease in ALB suggest a compromise in the integrity of hepatocyte membrane.[27] Research has shown that the liver is the prime site of As metabolism, and that arsenic species bind avidly to hepatocytes to cause injury and tissue damage as in our histology report.[10,28] In consistent with the previous studies,[29,30] As-induced hepatic injury may contribute to the extracellular outflow of ALT and AST into circulation in this study. The hepatic injury may reduce ALB levels, because maintenance of ALB in blood is related to the synthetic function of hepatic cells.[31] The effect of As on the hepatocytes might affect its synthetic function to undercut ALB production, leading to reduced level of ALB found in this study. Concomitantly, As exposure triggered nephrotoxicity shown by pronounced increases in creatinine and urea levels in this study [Table 2]. Creatinine and urea levels are diagnostic indicators of nephrotoxicity. The renal effect of arsenic toxicity includes loss of capillary integrity and glomerular dysfunction,[6] known to promote proteinuria, and reduced ultrafiltration of creatinine and urea which might have caused their increased serum levels and histological glomerular damage in this study.

Interestingly, we observed that VCO supplementation for 21 days in this study attenuated the As-induced hepatorenal toxicity. The biochemical indicators of liver and kidney damage were restored as well as histology significantly comparable to normal control. Although, to the best of our knowledge, this is the first study to report on benefit of VCO against As toxicity, accumulating evidence suggests beneficial effects of VCO in hepatorenal toxicity.[12,16] Furthermore, in a recent study by Nair *et al.*,[32] VCO supplementation reverses altered levels of ALT, AST, creatinine, and urea in a cyclophosphamide toxicity model. The health-promoting benefits of VCO have been attributed to its potent phytochemical contents.

Moreover, the mechanism for systemic toxicity of As has been associated with elevation of lipid peroxidation and depleted level of reduced glutathione.[6,30] The previous studies suggest oxidative mechanism for arsenic toxicity. Furthermore, experimental studies have reported that As binds to sulfhydryl proteins, including reduced glutathione to impair physiological antioxidant homeostasis.[33] Acute As administration prominently depressed antioxidant enzyme activities (SOD, CAT, and GPx), and consequently markedly increased MDA and NO levels. Abundant evidence demonstrates that As triggers excessive ROS generation known to cause oxidative stress status. However, oxidative stress depletes antioxidant defenses regulated by antioxidant enzymes, including SOD, CAT, and GPx.[30,33] Conceivably, our results, herein, suggest that As-induced oxidative stress reduced GPx, SOD, and CAT activities in this study. The depressed defense of antioxidant mechanism might promoted ROS attack on hepatorenal membrane for lipid peroxidation leading to increased MDA and NO levels in this study. Increased NO levels suggest that As triggers systemic inflammation. In cells, inflammation activates inducible nitric oxide synthase for the generation of NO. However, NO could react with other ROS to aggravate oxidative stress and/or impairs antioxidant system.[12] Our findings are in harmony with earlier reports that As promotes oxidative stress through impairment of SOD, CAT, and GPx activities and increased NO production.[3,33,34]

Increasing evidence shows that antioxidants may repress ROS and suppress lipid peroxidation. Accumulating research evidence suggests the presence of natural antioxidants in VCO.[13] In our study, it was observed that VCO supplementation inhibited suppression of SOD, CAT, and GPx activities initiated by As. In corollary, the inhibitory effect of VCO was further demonstrated through reduction in MDA and NO levels comparable to control animals [Figure 1]. These findings indicate that VCO restored antioxidant defenses against As-induced oxidative stress in rats. This is consistent with findings of previous studies that report on the VCO antioxidant activity that mitigated oxidative injury caused by antineoplastic drug,[12,32] antiretroviral agent,[15] and ethanol.[35] The phenolic compounds, including catechin, ferulic, *P*-coumaric, protocatechuic, vanillic, caffeic, syringic acid, and vanillic acids, have been suggested responsible for VCO beneficial health effects.[14]

Conclusively, as is a hepatorenal toxicant; its systemic toxicity is associated with damage to antioxidant mechanism and NO generation. Our study, hereby, shows that VCO could attenuates As-induced oxidative stress-mediated damage to liver and kidney through modulation of antioxidant enzymes, lipid peroxidation, and NO.

## FUNDING SOURCE DECLARATION

The authors of this manuscript self-funded the study.

## ACKNOWLEDGMENT

None.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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