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Original Research

Clerodendrum volubile and *Vernonia amygdalina* flavonoid fractions exhibit toxic metal chelation, microminerals, and thiol systems – augmenting potentials in arsenic exposed male rats

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Abstract

Exposure to arsenic from drinking water poses a significant threat to public health worldwide. *Clerodendrum volubile* and *Vernonia amygdalina* are potent natural sources of antioxidants to mitigate the toxic effect of arsenic. This study evaluated the effects of flavonoid fractions from *C. volubile* and *V. amygdalina* (FICV and FIVA) on the thiol cycling pathways and ion regulation of male albino rats exposed to sub-acute arsenic. Thirty male rats were randomly divided into six treatment groups. Control animals (distilled water), arsenic (40 ppm arsenic), arsenic + FICV (100 mg/kg), arsenic + FIVA (100 mg/kg), arsenic + FICV and FIVA (50 mg/kg each) and arsenic + Vitamin C (100 mg/kg). The treatment commenced four weeks after exposure to arsenic in drinking water and continued for a further four weeks. The liver and kidneys of the rats were excised following an overnight fast. Arsenic had caused significant (p<0.05) reductions in the total protein levels and metallothionein levels, reduced glutathione levels in the liver and kidneys, and decreased glutathione-S-transferase enzymatic activity. Additionally, essential elements (magnesium, zinc, copper and calcium) were significantly reduced (p<0.05) in the arsenic-exposed rats. Study results showed that the reductions were reversed after treatment with FICV and FIVA. This study concludes that flavonoid fractions from *C. volubile* and *V. amygdalina* possess potent therapeutic actions against arsenic-induced oxidative stress and toxicity in male albino rats.

Keywords: Essential elements, medicinal plants, arsenicosis, thiol systems, oxidative damage, chelation

Introduction

Arsenic is a naturally occurring metalloid. It is one of the top twenty most abundant elements in the earth's crust and a component of over 245 minerals. The inorganic form consists mainly of arsenic and arsenate compounds, which are toxic to human health [1]. Human exposure to arsenic primarily occurs through air, food and drinking water – most likely from arsenic-containing pesticides, natural mineral deposits or improperly disposed arsenical chemicals [1].

Chronic exposure to arsenic in drinking water can lead to carcinogenesis of almost all organs, including the skin and epithelial tissues, hepatic, renal, respiratory, central nervous system, gastrointestinal, and reproductive tissues [2]. Arsenic exposure also impairs intellectual reasoning in children, increases morbidity, decreases the quality of life and, ultimately, causes mortality in adults [3,2,1]. Trivalent arsenic toxicity can occur directly by attacking -SH groups or indirectly by generating reactive oxygen species (ROS) [4].

The thiol system, which includes glutathione and other thiol-containing systems, helps to maintain cellular redox balance and the ratio of reduced to oxidized glutathione indicates the cellular oxidative status [5] Metallothionein (MT) is a protein with many thiol-containing cysteine residues. It plays an important role in metal detoxification and is found in many tissues, including the liver, kidneys and brain. MT has also been shown to coordinate the metabolism and homeostasis of other essential metals like zinc (Zn), iron (Fe), copper (Cu), calcium (Ca) and magnesium (Mg) [6]. Owing to their extensive thiol system, the depletion of MT by heavy metals may predispose the body to oxidative stress [6]. Furthermore, the glutathione-S-transferase utilizes reduced glutathione as a co-substrate to conjugate and detoxify xenobiotics, which is impaired by arsenic exposure [7].

Cellular microelements, including iron, copper, zinc, calcium, and magnesium play essential roles in the body's metabolism, ranging from coordination of cellular proteins like haemoglobin by iron to serving as cofactors to many proteins and enzymes, such as copper in ceruloplasmin, zinc in alcohol dehydrogenase, and magnesium found in many kinases [8,9]. Disruption of the level of these elements poses significant harm to the body and might be an underlying mechanism for various diseases arising from arsenic toxicity [3,60]. Thus, treating arsenic toxicity requires subtle modulation of the body's antioxidant and elemental compositions.

The first step in the treatment of heavy metal poisoning is to eliminate the source of exposure and remove the individual from further exposure to the source, of which chelating agents are the most common therapy [10]. However, many treatments have limitations, including the inability to cross the blood-brain barrier and occasional adverse side effects, thereby necessitating a natural and non-toxic treatment regimen. Medicinal plants have been used since time immemorial for the treatment of ailments and have attracted the attention of researchers as potential treatments for heavy metal poisoning. Some herbs such as *C. nutans* and *H. diffussa* [11] contain metals chelating agents, which reduce heavy metals' bioavailability and gastrointestinal absorption. Moreover, some herbal therapies can decrease the bioavailability of toxic substances by increasing gastrointestinal movements, resulting in faster excretion of toxicants through the faeces [12].

Clerodendrum volubile is one species of the *Clerodendrum genus* [13]. This plant is popularly known in the southwest region of Nigeria [14]. Due to its medicinal properties, it is locally used to treat oedema, dropsy, and arthritis [15]. *Vernonia amygdalina* is another widespread plant grown throughout tropical Africa. It is locally known as 'bitter leaf' in Nigeria [16,17] and is tra-

ditionally used to treat diabetes, nausea, and dysentery [16]. It has been shown that these two plant extracts contain flavonoids from which their acclaimed antioxidative properties originate [16,17,18]. Due to the folklore use of these plants in treating many diseases associated with arsenic toxicity, we hypothesize that flavonoids from these plants might provide therapeutic treatment for arsenicosis. More information is needed on the potential effects of the flavonoid extracts of both plant extracts on arsenic-induced oxidative stress and cellular element perturbations in male rats. Thus, this study investigated the impact of FICV and FIVA on arsenic-intoxicated rats.

Materials and methods

Chemicals and reagents

Sodium arsenic ($NaAsO_2$), chloroform, methanol, n-hexane, dichloromethane, ethyl acetate, hydrochloric acid, sodium hydroxide, silica gel, sucrose, Tris Base, ethanol, EDTA, 5,5-dithiobis (nitrobenzoic acid), silica gel, n-hexane, sucrose, betamercaptoethanol, reduced glutathione, trichloroacetic acid, Folin-Ciocauteu's reagent, sodium carbonate, and copper sulfate pentahydrate used are products of Sigma Chemical Co (St. Louis, MO, USA). Methylated spirit and Vitamin C were obtained from PureChem Pharmaceuticals Nigeria Limited (Lagos, Nigeria); all other chemicals were of the pure grade available.

Plant collection and authentication

C. Volubile was obtained from a farm in Ijoko, Sango Ota, Ogun State, Nigeria, while *V. Amygdalina* was obtained from a domesticated farm in Odo-Eran, Abeokuta, Ogun State, Nigeria. Plants were collected at dawn, rinsed lightly with clean water, and air-dried under a shade. Dried samples were pulverized using a blender and kept in an airtight container for further use. Fresh samples were authenticated (*C. Volubile*; F101926; *V. Amgydalina*; F101863) at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria.

Isolation of flavonoids from plant materials

Extraction of the plants was done according to the process of Anila and Vijayalakshmi [19]. Briefly, pulverized plants were extracted with absolute methanol using the Soxhlet extraction method. They were then dried using a rotary evaporator, after which the extract was reconstituted in distilled water. Liquid-liquid extraction was then carried out in a separating funnel using n-hexane, n-hexane dichloromethane (70:30) and ethyl acetate. To 100 mL reconstituted extract, 100 mL of n-hexane was added and allowed to separate, then the n-hexane layer was decanted. The procedure was repeated using n-hexane: dichloromethane and ethyl acetate. The ethyl acetate fraction was allowed to evaporate to dryness. The ethyl acetate fraction was weighed, adsorbed on silica gel, and transferred to a column of silica gel equilibrated with n-hexane. Elution was carried out using chloroform: ethyl acetate (50:50), ethyl acetate (100%), ethyl acetate: methanol (75:25), ethyl acetate: methanol (50:50). In each case, 20 mL of fractions were collected in 100 tubes. Isolate was then concentrated using a rotary evaporator at 60°C.

Confirmatory test for flavonoid

A preliminary test for flavonoids was carried out on the ethyl acetate fraction. 1 mL of ethyl acetate fraction was put in a test tube and two drops of dilute sodium hydroxide solution were added. An intense yellow colour appeared, which became colourless with a few drops of dilute hydrochloric acid, indicating the presence of flavonoids [20]. The characteristics of the phytochemicals present in FIVA and FICV were shown in a previous study [21].

Biochemical assays

Total protein determination

Total protein was determined using a commercial kit from Randox Laboratories Limited (Crumlin, United Kingdom) [22].

Determination of Glutathione level

Glutathione levels in the liver and kidneys were determined using Ellman's spectrophotometric method [23], in which DTNB complexes with the thiol groups form a yellow complex measured at 412 nm.

Determination of Glutathione-s-transferase (GST) activity

Glutathione- S-transferase activity in whole blood was determined at 340 nm according to the method described by Habig and Jakoby [24] by measuring the formation of 1-chloro-2,4-dinitrobenzene and glutathione conjugate at 25°C and pH 7.0.

Determination of Metallothionein level

This was determined by Hala *et al*.'s method [25]. Tissue was placed in a homogenization buffer containing 0.5 M sucrose, 20 Mm Tris-HCl buffer, pH 8.6, containing 0.01% β-mercaptoethanol. The concentration of reduced sulfhydryl was evaluated by reading absorbance at 412nm.

Determination of heavy metals in blood and organs

The blood and organs were digested according to the method of Onunkwor *et al*. [26]. A 0.2 mL volume of each blood sample and 0.2 g of the organ were measured into boiling tubes to which 5 mL of concentrated nitric acid was added and digested using a water bath at a temperature of 80°C until a clear solution was obtained. The digest was then filtered into a 2 mL volumetric flask and made up to mark with 5% (v/v) nitric acid. The solutions were stored in acid-washed plastic tubes until ready for analysis for metals. Heavy metal analysis was carried out using inductively coupled plasma-optical emission spectrometry (ICP-OES).

Statistical analysis

Results were analyzed using one-way ANOVA, followed by Tukey's test for comparison among the groups (p<0.05). Graphs were plotted using Graph Pad Prism (v. 8.0).

Results

Effects of FICA, FICV and Vitamin C on the antioxidant system of arsenic-exposed rats

The effects of FICV, FIVA, and Vitamin C on liver and kidney health amidst arsenic exposure were examined. The effects of FICA, FICV and Vitamin C on the antioxidant system of arsenic-exposed rats are evident in **Fig. 1a-h**. Results showed that arsenic-exposed groups exhibited a staggering depletion of total protein (TP) levels in the kidney and liver, plummeting by 22.8% and 88.4%, respectively, compared to the control group (p<0.05). However, treatments including FIVA, FICV, FICV+FIVA and Vitamin C resulted in a remarkable recovery, augmenting the depleted TP levels in the kidney by 107.2%, 65.2%, 94.3%, and 118.1% respectively as shown in **Fig. 1a**. The liver shows a similar trend with increments of 34.1%, 193.2%, 104.8%, 125.6%, and 147.4%, respectively, recorded against the arsenic group as seen in **Fig. 1e**.

Figs 1b and **1f** depict the reduced glutathione (GSH) levels in arsenic-exposed kidneys and livers. GSH levels declined by 79.5% and 41.3% in the arsenic group. Treatments with FIVA, FICV, FICV+FIVA, and Vitamin C showcased a remarkable recovery of GSH in the treated rats following arsenic exposure. Notably, the liver exhibited increases of 40.5%, 8.6%, 70.7%, and 101.9%, respectively, compared to the arsenic group.

In **Figs. 1c** and **1g**, the specific activity of GST in the kidneys and livers of arsenic-exposed groups was compared to the control group. A significant decrease (p<0.05) in GST activities was evident in both the kidneys (54.27%) and the liver (73.3%) compared to the control group. However, interventions with FIVA, FICV, FICV+FIVA, and Vitamin C showcased compelling outcomes in the kidney. Substantial increments (p<0.05) of 322%, 48%, 786%, and 320% were observed compared to the arsenic group. In the liver, treatment restored GST activity to normal levels. Notably, arsenic-intoxicated rats treated with FICV and Vitamin C did not exhibit significant differences (p>0.05) compared to the arsenic group. Meanwhile, FIVA and FIVA+FICV-treated groups displayed substantial decreases (p<0.05) of 104.6% and 59.0%, respectively, compared to the arsenic group.

Figs. 1d and **Fig. 1h** show the levels of MT in the kidneys and livers of arsenic-exposed rats compared to the control group. The untreated arsenic group showed significant reductions (p<0.05) of 57.2% and 62.7%, respectively. Remarkably, treatment with FIVA, FICV, FICV+FIVA, and Vitamin C yielded substantial increases (p<0.05) in MT levels in the kidney by 368.3%, 33.9%, 68.9% and an impressive 383.0% respectively, compared to the arsenic-exposed group. In the liver, similar trends were observed with increases of 80.4%, 143.5%, 107.1%, 171.3%, and 148.6% respectively compared to the untreated arsenic group.

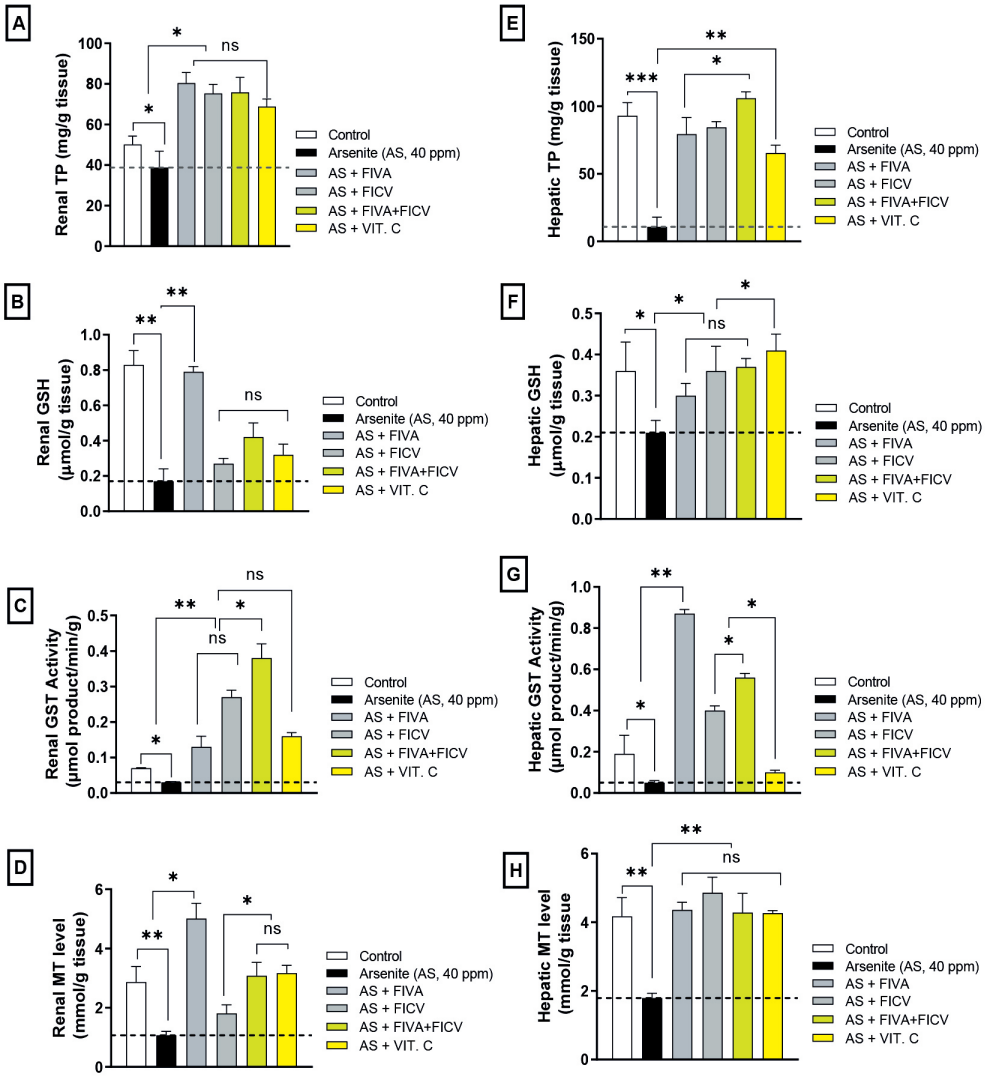

Fig. 1: ***Effects of flavonoid-rich extracts from C. volubile (FICV), V. amygdalina (FIVA), and Vitamin C (Vit. C) on hepatorenal antioxidant status and total protein level in rats exposed to arsenic.***
Values represent mean ± standard error of the mean (n=5).
Bars with different letters differ significantly (*p<0.05; **p<0.001; ***p<0.0001).
(A) Renal total protein (TP) level;
(B) Renal reduced glutathione (GSH) level;
(C) Renal glutathione-S-transferase (GST) activity;
(D) Renal metallothionein (MT) level;
(E) Hepatic total protein (TP) level;
(F) Hepatic reduced glutathione (GSH) level;
(G) Hepatic glutathione-S-transferase (GST) activity;
(H) Hepatic metallothionein (MT) level.

Metal composition (mg/100 g)	*C. volubile*	*V. amygdalina*	*p*-value
Arsenic ($\times 10^{-3}$)	0.56 ± 0.05	0.21 ± 0.02	0.02*
Zinc	25.28 ± 0.76	28.17 ± 1.75	0.205
Copper	2.18 ± 0.18	1.85 ± 0.06	0.152
Calcium	31.77 ± 0.83	30.73 ± 0.61	0.368
Magnesium	28.05 ± 0.04	28.82 ± 0.66	0.31
Crude fibre	11.39 ± 0.34	12.66 ± 0.25	0.040*
Fat	5.16 ± 0.19	6.23 ± 0.08	0.007**
Carbohydrates	41.41 ± 0.68	49.66 ± 1.15	0.003**

Table 1: Proximate analysis of *C. volubile* and *V. amygdalina*

Table 1 shows a. *V. amygdalina* had the highest carbohydrate content (49.66 ± 1.15%) and moisture (18.43 ± 0.56%), while *C. volubile* had the highest ash content (17.84 ± 1.01%) and crude protein (12.64 ± 1.09%) contents. Also, the elemental composition of the plants, *V. amygdalina* had a higher concentration of zinc (28.17 ± 1.75 g/100 g) and magnesium (28.82 ± 0.66 g/100 g), while manganese (31.77 ± 0.83 mg/100g) was higher in *C. volubile*.

Fig. 2 depicts the zinc and copper concentrations in the kidneys and liver of animals when compared with the control and arsenic-exposed groups. Exposure to arsenic led to a significant (p<0.05) decrease in zinc and copper concentrations in both organs, highlighting the impact of arsenic on these elements. When comparing the arsenic group with the treated groups, zinc concentration significantly increased in all treatment groups except the kidneys, where only the FIVA and Vitamin C treatments showed substantial increments. Notably, no significant change in zinc concentration was observed in the FICV and FIVA+FICV treatment groups compared to the arsenic-exposed group. Conversely, significantly increased copper concentrations were observed in both the kidneys and livers of the treated groups compared with the arsenic group. On the one hand, FIVA had the highest impact on zinc levels in the kidney, while FIVA and FICV exhibited similar effects in the liver. On the other hand, the liver of rats treated with FIVA showed the highest copper levels.

Fig. 2 also provides insights into the calcium and magnesium concentrations in arsenic-exposed kidneys and liver treated with FIVA, FICV, FIVA+FICV and Vitamin C. A significant decrease (p<0.05) in calcium concentration was noted in the arsenic-exposed control group compared to the control group. However, when comparing the arsenic group with the treated groups, there was a notable increase in calcium and magnesium concentrations in both organs across all treatment groups, signifying the efficacy of the treatments in elevating these elements' levels.

Figs. 3a and **3b** showcase arsenic levels in the kidneys and liver post-arsenic exposure and subsequent treatment. Compared to the control, the arsenic group had a significantly increased arsenic concentration. However, after treatment, a remarkable decrease in arsenic concentration was observed in both the kidneys and liver of groups exposed to arsenic and treated with the different regimens. Vitamin C exhibited the most profound effect in reducing arsenic levels in the kidneys, while all treatment regimens showed equal efficacy in reducing arsenic levels in the liver.

Data are expressed as mean +/- SEM (taken in triplicates). The mean elemental compositions between *C. volubile* and *V. amygdalina* were compared using an unpaired student's t-test (*p<0.05; **p<0.001).

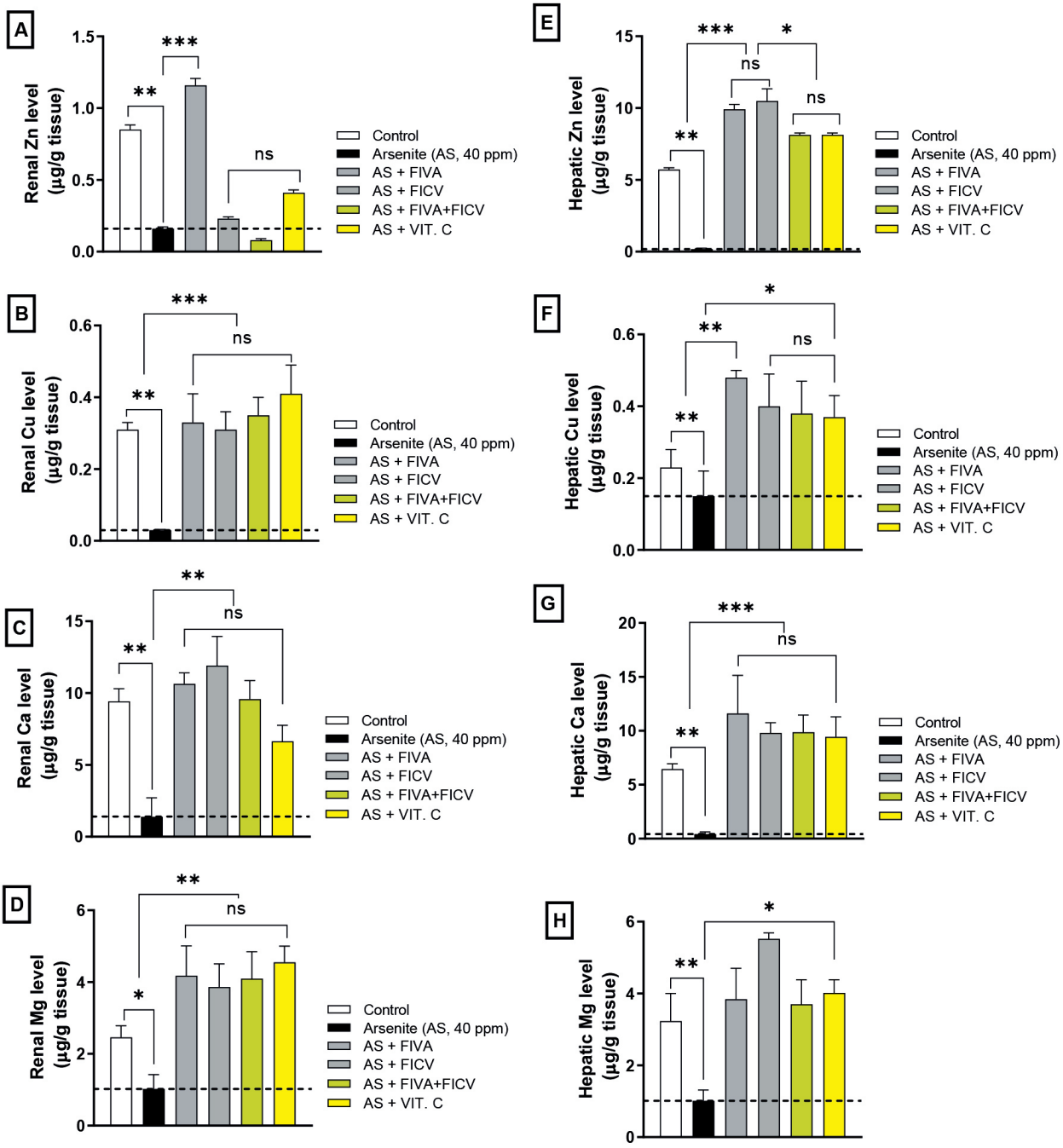

Fig. 2: Effects of flavonoid-rich extracts from *C. volubile* (FICV), *V. amygdalina* (FIVA), and Vitamin C (Vit. C) on hepatorenal essential elements dynamics in rats exposed to arsenic.

Values represent mean ± standard error of the mean (n=5). Bars with different letters differ significantly (*p<0.05; **p<0.001; ***p<0.0001). (A) Renal zinc (Zn) level; (B) Renal Copper (Cu) level; (C) Renal Calcium (Ca) activity; (D) Renal Magnesium (Mg) level; (E) Hepatic Zinc (Zn) level; (F) Hepatic Copper (Cu) level; (G) Hepatic Calcium (Ca) activity; (H) Hepatic Magnesium (Mg) level.

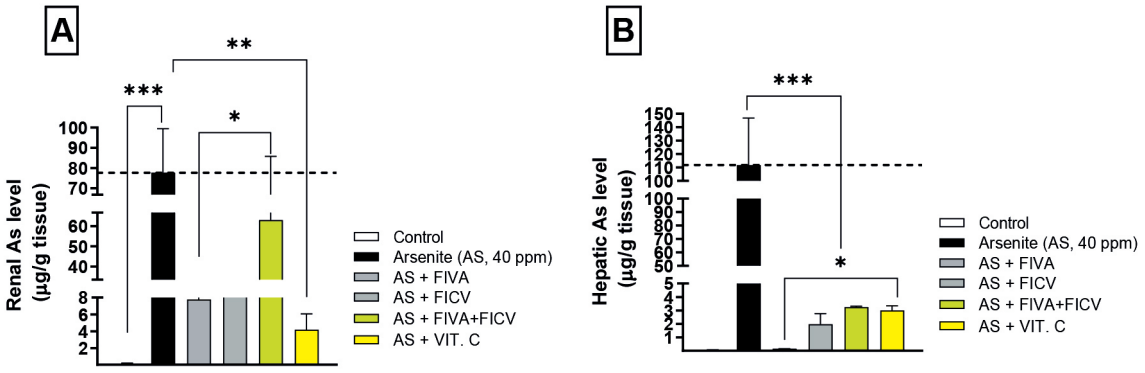

Fig. 3: Effects of flavonoid-rich extracts from *C. volubile* (FICV), *V. amygdalina* (FIVA), and Vitamin C (Vit. C) on hepatorenal arsenic levels in rats exposed to arsenic.

Values represent mean ± standard error of the mean (n=5). Bars with different letters differ significantly (*p<0.05; **p<0.001; ***p<0.0001). (A) Renal arsenic (As) level; (B) Hepatic arsenic (As) level.

Discussion

Arsenicosis, prevalent in areas of water contamination and coal combustion areas, poses severe health risks, damaging vital internal organs and systems [27,28,29,30]. Chronic arsenic exposure can harm the respiratory, digestive, circulatory, neural, and renal systems [31,32]. Treatment methods have varied from chelation therapy to allopathic drugs and synthesized chemicals like British Anti-Lewisite antidote (BAL) and dimercaptosuccinic acid (DMSA), which are not readily available, expensive and may produce a range of adverse effects. As a result, the focus on arsenicosis treatment has shifted towards medicinal plants that have the potential to mitigate arsenic-related ailments. However, understanding the active phytochemicals responsible for these beneficial actions remains a quest [33,34]. This study dives into the impact of flavonoid-rich extracts from *C. volubile* and *V. amygdalina* on the cellular thiol antioxidant system in male rats exposed to sub-chronic arsenic levels.

In this investigation, arsenic exposure triggered a significant (p<0.05) decline in TP levels within the liver and kidneys of exposed male albino rats compared to the control group. Thiol, a vital component in compounds like glutathione and MT, is a linchpin in the cellular antioxidant system, crucial for neutralizing internally generated free radicals. This depletion of TP hints at disrupted protein synthesis due to arsenic accumulation, echoing findings in previous studies [35, 36].

The liver's role in xenobiotic transformation and the kidneys' functions in waste elimination and metabolic balance expose these organs to heavy metal toxicity. Arsenic's known mechanism of inhibiting antioxidant and xenobiotic-detoxifying enzymes reliant on thiol groups elucidates the reduced protein levels observed in this study [37,38]. The irreversible binding of metabolized arsenicals to liver and kidney proteins, specifically targeting thiol groups, likely contributes to this decline. Moreover, prior research highlighting the potential of *V. amygdalina* to counteract arsenic-induced toxicity due to flavonoids and polyphenols adds depth to our understanding [39]. Importantly, this study pioneers the investigation into *C. volubile's* mitigating effects on arsenic-induced oxidative stress in rats. The flavonoid fraction of *C. volubile* (FICV) emerged with the remarkable ability to boost TP levels.

This enhancement is linked to the antioxidant prowess of flavonoids, attributed to their structural characteristics and the presence of hydroxyl groups that adeptly scavenge free radicals, thereby curbing oxidation within the body [40,41]. In this study, arsenic caused a significant (p<0.05) decrease in the MT levels in rats' liver and kidneys compared with the control group. MT is made up of thiol (-SH group) and other thiol-containing proteins. There may be an increase in the MT level at the onset of oxidative stress due to an adaptive homeostatic response of the cell, however, when the oxidative stress attains an overwhelming degree, the thiol group in MT decreases significantly due to the inability to replenish its pool [42].

Considering the affinity of heavy metals such as arsenic for thiol-containing compounds, a decrease in MT levels in the groups exposed to arsenic was observed. The lowered level of thiol content following arsenic exposure is consistent with other studies [43]. Flavonoid fractions of *C. volubile* and *V. amygdalina*, either singly or combined with Vitamin C, reversed the decrements in MT levels.

Glutathione, the most abundant tripeptide thiol, exists as GSH (reduced form) in cells and participates in several physiological processes, including detoxification of xenobiotics. Primarily, it catalyzes disulphide exchange reactions. It is facilitated by scavenging free radicals, detoxifying xenobiotics, and oxidizing itself to GSSG [44]. Besides its involvement in the detoxification process, GSH also plays a vital role in lymphocyte function. Depleting GSH content was associated with impaired immune response and increased risk of malignancy [45]. As previously discussed, arsenic has a high affinity for sulfhydryl groups; hence, it actively binds with reduced glutathione, causing its depletion in tissues. When GSH levels are depleted, the arsenicals accumulate in the system, increasing toxicity [46]. In this study, treatment with *C. volubile*, *V. amygdalina*, and Vitamin C resulted in significant recovery of GSH concentration in arsenic-intoxicated rats. The improved levels of GSH protected the sulfhydryl groups from binding with arsenic. They promoted the detoxification of arsenic by modulating arsenic methylation reactions, favouring its excretion from the system [47].

GST consists of a large family of GSH utilizing enzymes that play an essential role in detoxifying xenobiotics in mammalian systems. Arsenic trioxide inhibited GST activity significantly in the liver and kidney [19,48]. However, ascorbate co-treatment restored enzyme activity in male rats. This was considered an adaptive response facilitated by ascorbic acid against arsenic-induced stress. Reduced glutathione is responsible for the intrinsic antioxidant system of mammalian cells. This study showed that relatively high arsenic levels and the prolonged exposure significantly decreased GST activity in the organs. This suggests that arsenic made GST unavailable because of the formation of the arsenic-GST complex [49]. Remarkably, FIVA and FICV abolished the arsenic-induced oxidative stress by augmenting the antioxidant enzymes' activities singly or in combination. Although the treated group showed better enhancement in GST activity, the GST activity increased significantly following treatment with both flavonoid fractions, without any significant difference between either plant. This ability to quench superoxide radicals and modulate enzymatic activities by flavonoids was reported by Kulshrestha [38], emphasizing our observations. This study provides a mechanism by which disruption of the body's antioxidants could lead to perturbation of the essential elements during arsenic toxicity. Many divalent elements, including zinc, iron, copper, and magnesium, play diverse homeostatic and metabolic roles in the body, and their concentration can be impaired by arsenic and other toxic metals [1].

Zinc is the body's second most crucial trace element after iron [50]. It participates in

many metabolic processes, possessing three major biological roles as a structural, catalytic, and regulatory component [51]. Zinc is an essential trace element in human health, and its deficiency can lead to retarded growth, anorexia, smell and taste failure, and other symptoms in humans [52]. It mediates its antioxidant effect directly and indirectly. It binds to the thiol and sulfhydryl groups in proteins and peptides; zinc inhibits the production of reactive species oxidation, thus stabilizing the cell membrane [53,54]. The roles of zinc in detoxifying arsenic have been reported but with contradictory outcomes. Most studies support that zinc protects against arsenic toxicity by restoring the prooxidant-antioxidant balance, increasing GSH, activating antioxidant enzymes, or inducting MT synthesis [55,56]. This study showed that treatment with flavonoids from *C. volubile* and *V. amygdalina* restored the antioxidant balance by activating antioxidant enzymes and increasing zinc concentration.

Copper is an essential trace element in humans and animals. Most of the 100 mg of copper in the human body is a cofactor in ceruloplasmin, a copper-dependent ferroxidase enzyme with oxidation activity [57]. Coupled with its role in iron metabolism, the need for copper also derives from its involvement in many biological processes, including antioxidant defence, neuropeptide synthesis and immune function [58]. Though an essential micronutrient, excessive copper is highly toxic via the Fenton-type redox reactions, resulting in oxidative cell damage and cell death. However, copper toxicity due to dietary excess is generally not considered a widespread health concern, probably due to the homeostatic mechanisms controlling copper absorption and excretion [57]. In utero, copper deficiency may impair cardiovascular system development, bone malformation and ongoing neurologic and immunologic abnormalities into infancy and beyond [58]. In adulthood, prolonged marginal copper deficiency has been associated with alterations in cholesterol metabolism [59]. It is important to note that there needs to be more information on the effect of copper on other heavy metals. In this study, arsenic hindered the amount of dietary copper available for normal functioning of the body's organs. This shows that flavonoid fraction from *V. amygdalina* and *C. volubile* could reverse the depletion of Cu owing to arsenic toxicity. The mechanism underlining the recovery is unclear and warrants further investigation.

Calcium is vital for life and is the most abundantly stored nutrient in the human body; more than 99% (1.2–1.4kg) is stored in the bones and teeth and about 1% is found in extracellular serum calcium. Calcium participates in different physiological processes, cell signalling and bone growth [59]. Muscle function, nerve activity, and bone mineralization depend on a precise extracellular and intracellular calcium balance. Zhang *et al*., (2013) reported a possible dose-dependent relationship between calcium supplement levels and the reduction of blood lead levels. [60] Arsenic, as a heavy metal, can suppress the effect of other essential metals, as seen in this study; it achieves this by rendering critical metals unavailable for use. Although treatment with plants reversed this effect, this can be attributed to essential metals in the plants, as shown in the proximate and elemental analysis of the plants.

Magnesium plays many roles in the human body, including its cofactor for more than 300 enzymes. It regulates cellular functions like muscle contraction, neuromuscular conduction, glycaemic control, myocardial contraction, and blood pressure [61]. Furthermore, magnesium also plays a vital role in energy production, active transmembrane transport for other ions, synthesis of nuclear materials, and bone development [62,63]. Magnesium acts as a counter ion for the energy-rich ATP and nucleic acids, regulates transmembrane transport and

has various roles in the function and structure of proteins, nucleic acid, and mitochondria [64]. Magnesium deficiency might induce oxidative stress (characterized by an increased level of lipid peroxidation) and hypertension [65,66,67].

Arsenic is reported as an active species that reacts with biomolecules like lipids, proteins, and DNA, thereby impairing their functional properties. This causes alterations in the normal everyday function of cells, tissues, organs and ultimately organisms evident as disease symptoms, and other pathological conditions [67]. The involvement of reactive oxygen species in metal-induced cell death is widely reported. Strategies to limit or abrogate heavy metals-induced free radical challenges on iron could be through the inhibition of H2O2 formation, chelation of ferrous ions, or trapping of the radicals formed [66]. The thiol compound is the most effective compound to protect cells against heavy metals-induced toxicity. Reduced glutathione levels decreased in the arsenic-exposed group. This suggests that arsenic may have made GSH unavailable because of the formation of the arsenic-GSH complex [49]. Remarkably, FICV or FIVA abolished arsenic-induced oxidative stress by augmenting the antioxidant enzymes' activities singly or in combination. However, this ability to quench superoxide radicals and modulation of enzymatic activities by flavonoids is well documented elsewhere [34]. This could be due to their structures' multiple hydroxyl groups (electron-donating groups) [34,35]. Therefore, the flavonoid fractions from *C. volubile* and *V. amygdalina* may abolish oxidative stress invoked by arsenic exposure in rats due to their enormous *in vivo* antioxidant effects. Data from our study suggests that flavonoids derived from *C. volubile* and *V. amygdalina* possess tremendous bioactivities against oxidative stress-related complications.

Further studies focusing on the exact flavonoid(s) responsible for these effects are required to elucidate the molecular target of such phytochemicals(s). This study has limitations that necessitate further studies. The lack of an exact molecular mechanism in the tissues investigated means that our observations only serve as a baseline for mechanistic studies to fully unravel the underlying mechanisms through which FIVA and FICV primarily could mitigate ion disturbances.

Conclusion

This study validates the augmenting effects of FICV and FIVA against oxidative damages levied by arsenic exposure. This mechanism is via the attenuation of oxidative stress indices and the concomitant enhancement of the antioxidant molecules/enzymes singly or in combination. The observed effects are likely due to electron-donating sites in their flavonoid structures that quench radicals' production or their ability to modulate the activity of the antioxidant enzymes. Therefore, flavonoid fractions from *C. volubile* and *V. amygdalina* could be a feasible weapon against arsenic-induced hepato-renal oxidative stress and microelements disruptions in rats.

Funding

None.

Conflict of interest

The authors have no actual or potential conflict of interest that could inappropriately influence or be perceived to influence their study.

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