



Alleviation of the arsenic induced hepatotoxicity in rats by ginger or omega-3: a histological and biochemical study

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ABSTRACT

Background: Millions of individuals worldwide are unintentionally exposed to arsenic through food and water. Arsenic bioaccumulates in the human body and causes organ toxicity, especially hepatotoxicity, through apoptosis, inflammation, and the production of oxidative stress markers.

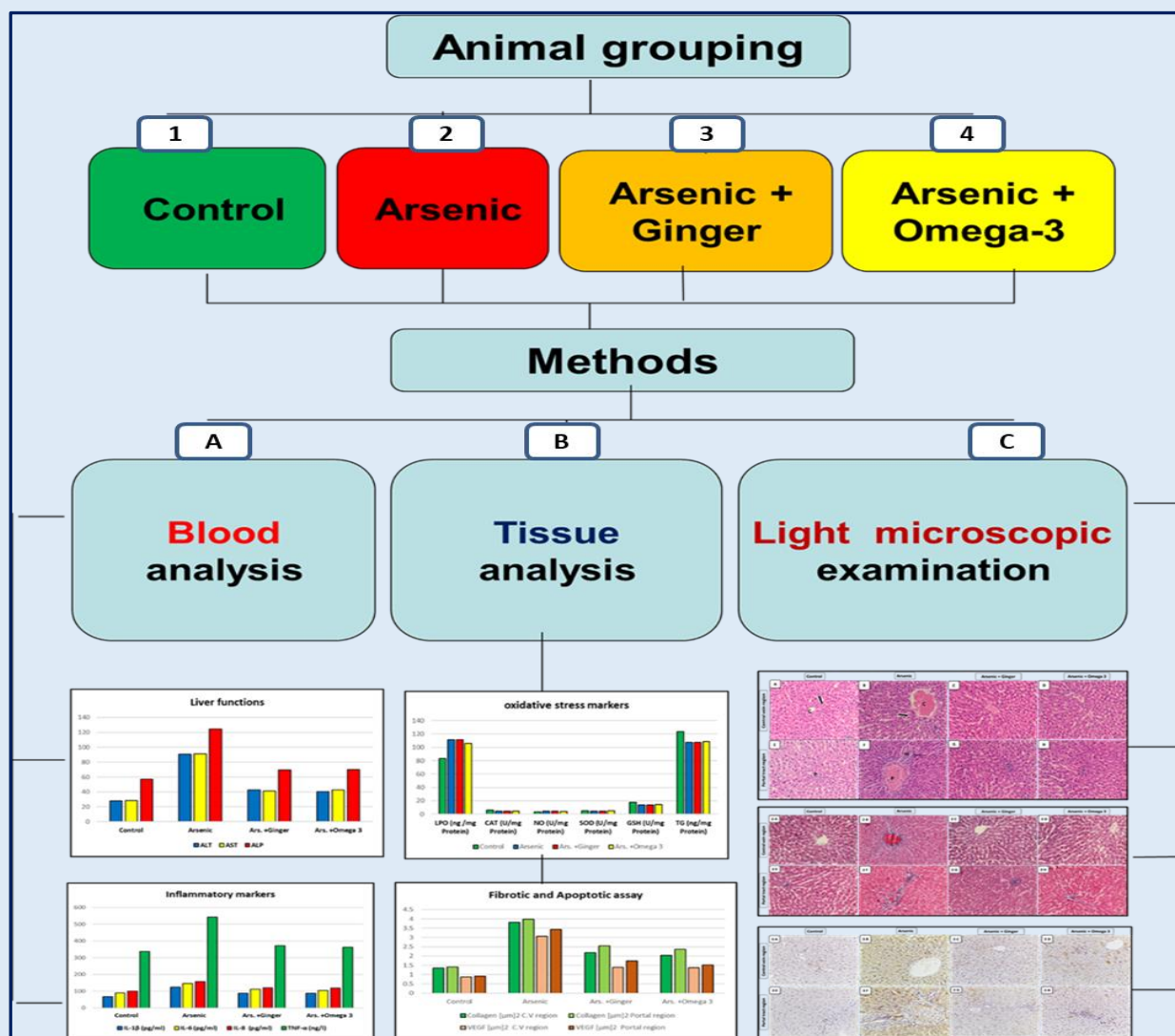
Aim of the study: We aimed to determine whether Ginger (*Zingiber officinale*) or omega-3 could mitigate the hepatotoxicity that rats experienced from long-term exposure to arsenic in drinking water.

Methods: Rats were randomly assigned into four groups (10/group): a control group, an arsenic exposed group (20 mg/kg body weight) only, or an arsenic exposed group in the presence of either *Zingiber officinale* (12 mg/kg of body weight) or omega-3 (100 mg/kg b.w.) daily for 28 days. We used different biochemical parameters to assess liver functions, such as oxidative stress parameters, inflammatory markers, and histological and immunohistochemical stains.

Results: Arsenic exposure to rats in drinking water results in hepatotoxicity, proved by increased liver enzymes and structural pathological changes in the liver, those mediated by increased oxidative stress, antiapoptotic effects, and inflammatory parameters, while supplementation of either *Zingiber officinale* or omega-3- to arsenic exposed rats significantly ($P < 0.05$) improved liver function biomarkers and alleviated oxidative stress induced by arsenic as well as, liver protection as confirmed by our histological and findings.

Conclusion: Ginger or omega-3 are safe and effective antioxidants to ameliorate arsenic-induced hepatotoxicity.

Keywords: Arsenic; Oxidation; Inflammation; Ginger; Omega-3.



INTRODUCTION

The existence of life on Earth depends mostly on water, and both people and the ecosystem need access to clean water [1]. However, over the past few decades, factors like a rising population, rapid industrialization, growing urbanization, and irresponsible use of natural resources have negatively impacted water quality [2].

Global health is seriously threatened by pollution of the air, water, and soil [3]. According to the Lancet Commission on Pollution and Health research, pollution is currently the primary global cause of disease and premature mortality due to the environment. In 2015, pollution-related diseases were projected to have caused 9 million premature deaths, or 16% of all deaths worldwide, and three times more common than tuberculosis, AIDS, and malaria combined [4].

Arsenic (As) is one of the most prevalent environmental toxins in the world. It is also one of the most significant water pollutants, representing a public health risk, with 783 million people lacking access to safe water worldwide [5]. The maximum acceptable concentration level (MCL) of arsenic in drinking water has been set at 10 µg/L by the World Health Organization, the US Environmental Protection Agency, and the European Union [6, 7].

Prolonged exposure to high levels of arsenic in drinking water has detrimental effects on health, such as cardiac problems, diabetes, lung and liver diseases, and even cancers [8]. As it plays a crucial role in the metabolism and detoxification of poisons, the liver is thought to be the optimum location for arsenic-induced fatal consequences [9]. The mechanism of arsenic-induced hepatotoxicity is still unclear, but according to the currently available data, inflammation and oxidative stress could be implicated in the tissue damage caused by arsenic [10]. Additionally, rodent-based experimental research showed that Arsenic mediated hepatic damage in a rodent model is linked to the onset of oxidative stress and apoptosis [11].

Thus, effective antioxidative or chemo-preventive methods are needed to lessen the negative effects of Arsenic on the population that is exposed. Numerous treatment methods by natural products have been devised to alleviate the harmful effects of heavy metals [12]. Functional foods are growing because of customers' preference for goods that offer more than just essential nourishment and their growing health consciousness. They are rich in biologically active compounds that, when consumed in certain, safe doses, offer a documented and clinically proven health benefit by lowering the risk of chronic and viral diseases and managing their symptoms [13-14].

Omega-3 is a polyunsaturated fatty acid mainly acquired through diet since the body cannot synthesize enough [15]. Several studies have demonstrated the safety of administering omega-3 fatty acids through fish oil supplements, which also show anti-inflammatory and antioxidant properties by inhibiting proinflammatory cytokines and oxidative stress [16-17].

Also, ginger (*Zingiber officinale*) is one of the most widely used food spices among Asians and Africans [18]. Because of its active components, ginger is regarded as a safe medicinal herb that has antioxidative, antiemetic, anticancer, anti-inflammatory, and antihyperglycemic effects in studies on several organs [19-22].

To our knowledge, no research has compared the alleviation of arsenic-induced hepatotoxicity in rats by ginger or omega-3 using different parameters; also, no study has analyzed the fibrotic and apoptotic changes using an image-analyzing microscope.

Therefore, we analyzed the effect of arsenic on liver architecture and function and assessed the possible protecting role of either *Zingiber officinale* or omega-3 Supplementation with arsenic exposure in rats through histological, immunohistochemical, and biochemical parameters.

METHODS

Chemicals

Sodium arsenite: We purchased Sodium arsenite (Na_2HAsO_4) as a white powder from Sigma-Aldrich (St. Louis, Missouri, USA). Animals received Sodium arsenite (AS) dissolved in normal saline (0.9% NaCl) orally by gastric gavage at a dose of 20 mg/kg body weight (BW) [23].

Zingiber officinale (Ginger): We produced and chilled the ginger watery extract at 4°C after purchasing fresh ginger roots (*Zingiber officinale* Roscoe) from the nearby market. Following that, it was given orally by gastric gavage at a dose (12.5 mg/kg bw) daily by oral gavage [24].

Omega-3 (fatty acids): It was derived as a soft gelatin capsule (SEDICO Pharmaceutical Co., Egypt). Each capsule contains fish oil-1000 mg (13% eicosapentaenoic acid (EPA) and 9% docosahexaenoic acid (DHA)). Rats were given a dose of (100 mg/kg b.w) once daily by oral gavage [25].

Animals: Forty adult male albino rats (eight weeks old) weighing 120–150 grams (gm) were provided by the National Research Centre (Giza, Egypt). The animals were housed at 22 ± 3 °C in hygienic plastic cages with a 12-hour light/dark cycle and access to normal pellet food and water. The rats were allowed to get used to this environment for seven days before the study began.

Experimental design and drug administration: After a week of acclimatization, the animals were randomly assigned into four groups of ten rats (5 per cage). Every group was administered its dose by oral gavage once a day for 28 days and fed with standard pellet food:

Group 1 (control) rats received distilled water; Group 2 (AS. only) rats received a daily dose of Sodium arsenite at 20 mg/kg body weight dissolved in normal saline; Group 3 (AS. + ginger): rats received a daily dose of Sodium arsenite at 20 mg/kg body weight dissolved in

normal saline plus ginger (12 mg/kg of body weight) in normal saline; Group 4 (AS. + Omega-3), rats received daily dose of Sodium arsenite at 20 mg/kg body weight dissolved in normal saline plus Omega-3 (100 mg/kg b.w).

Blood collection and preparations: At the end of the trial's study (on day 28), blood samples were obtained from the retro-orbital plexus following scarification by cervical decapitation, and serum was extracted by centrifugation at 1200 g for 15 minutes. The serum was then collected and stored at 20 °C for additional biochemical calculations.

Serum biochemical analyses: We assayed the serum levels of alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). We also measured serum proinflammatory interleukins: IL-1 β , IL-6, IL-8, and tissue necrosis factor alpha (TNF- α). The results were calculated using commercial Biodiagnostic kits (Giza, Egypt) according to the manufacturer's protocol.

Liver tissue samples: The liver of each rat from each group was excised into small samples (1-2 cm), cleaned, and then split into two samples. The first sample was homogenized in phosphate buffer saline (0.1 M PBS with pH 7.4) and stored at -80 °C until it was needed for measurements of antioxidant enzyme activity and oxidative stress catalase (CAT), reduced glutathione (GSH) value, lipid peroxidation (LPO), nitric oxide (NO), total glutathione (TG) and superoxide dismutase (SOD) were also measured [26]. The second liver tissue sample was fixed in 10% formol saline solution, dehydrated in ethanol, cleared in xylol, embedded in paraffin, sectioned by microtome (4-6 μm), and stained with either hematoxylin and eosin (H&E) for structural changes or Masson trichrome for fibrotic changes and immunohistochemical stain for apoptotic changes [27].

The prepared slides underwent light microscopy examination. Using a Raywild E5 microscope with a built-in digital camera (M-300) and image-analyzing software

(Mvi-mage program v12), pictures were captured, and the density of collagen fibers percentage area and immune expression of vascular endothelial growth factor (VEGF) were quantified.

Ethical approval and animal handling: All animal procedures followed the international guidelines for the care and use of animals for scientific purposes, and the study protocol was authorized under Approval No. 0001267-23-09-020 by the medical ethics committee of the Damietta Faculty of Medicine, Al-Azhar University, Egypt.

Statistical analysis: Processing and gathering of data were completed. The mean ± SE was used to depict the

data. The statistical package for social sciences (SPSS/version 21) application performed the one-way ANOVA. Duncan's post hoc test was utilized for multiple group comparison, and statistical significance was assessed at the level of P<0.05.

RESULTS

Liver functions: Blood results from rats exposed to drinking water containing arsenic showed elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and serum alkaline phosphatase (ALP). Whereas groups that received arsenic plus Ginger or Omega 3 showed a substantial decrease (P <0.05) in those parameters (Table 1)

Table (1): Analysis of serum liver enzyme levels in the various study groups.

Parameters	Control	Arsenic	Ars. +Ginger	Ars. +Omega 3
ALT [U/L]	27.9±3.81	90.6 ± 4.95 ^a	42.7 ± 5.62 ^b	40.3±3.89 ^b
AST [U/L]	28.2±3.97	91.3±3.4 ^a	41.4±3.37 ^b	42.8±5.14 ^b
ALP [mg/dl]	57.1±5.78	124.6±8.21 ^a	69.4±5.84 ^b	70.2±6.25 ^b

ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: alkaline phosphatase; Ars.: Arsenic ^asignificant difference between the arsenic and control groups; ^bsignificant differences between arsenic plus Ginger or Omega 3-treated groups and the Arsenic group.

Oxidative stress parameters: Tissue levels of Lipid peroxidation (LPO) and nitric oxide (NO) levels were elevated in rats exposed to drinking water containing arsenic. In contrast, the levels of superoxide dismutase (SOD) activity, catalase (CAT) activity, reduced

Glutathione (GSH) and total glutathione (TG) were found to be decreased following exposure to arsenic in the liver, compared to those levels in the control group. Whereas the groups that received arsenic plus Ginger or Omega 3 showed a significant change (P <0.05) in those parameters (Table 2).

Table (2): Analysis of oxidative stress level markers in the various study groups

Tissue parameters	Control	Arsenic	Ars. +Ginger	Ars. +Omega 3
LPO (ng /mg Protein)	83.39±7.01	161.31±14.02 ^a	111.44±6.67 ^b	105.92±4.88 ^b
CAT (U/mg Protein)	6.12±1.12	2.27±0.34 ^a	4.47 ± 0.19 ^b	4.51 ± 0.51 ^b
NO (U/mg Protein)	3.55±0.37	5.45±0.41 ^a	4.18±0.24 ^b	4.17±0.20 ^b
SOD (U/mg Protein)	5.23±0.39	3.12±0.31 ^a	4.58±0.53 ^b	4.91±0.31 ^b
GSH (U/mg Protein)	17.9±0.40	6.91±0.25 ^a	13.96±0.31 ^b	14.2±5.36 ^b
TG (ng/mg Protein)	123.7±7.86	86.16±6.55 ^a	107.39±3.89 ^b	108.89±3.92 ^b

LPO: Lipid peroxidation; CAT: catalase; No; nitric oxide; SOD: Superoxide dismutase; GSH: reduced glutathione; TG: total glutathione; ^asignificant difference between the arsenic and control groups; ^bsignificant differences between arsenic plus Ginger or Omega 3-treated groups and Arsenic group.

Inflammatory markers levels: Rats exposed to arsenic had higher serum levels of pro-inflammatory cytokines, IL-1 β , IL-6, IL-8, and tissue necrosis factor alpha (TNF- α) than the control group. Whereas the groups that

received arsenic plus Ginger or Omega 3 showed a significant decrease (P <0.05) in those parameters (Table 3).

Table (3): Analysis of levels of serum inflammatory markers in the various study groups

Parameters		Control	Arsenic	Ars. +Ginger	Ars. +Omega 3
IL-1 β (pg/ml)		67.09 \pm 8.83	124.28 \pm 12.68 ^a	87.68 \pm 7.83 ^b	86.66 \pm 6.24 ^b
IL-6 (pg/ml)		90.13 \pm 5.53	146.18 \pm 13.37 ^a	110.44 \pm 5.94 ^b	104.47 \pm 3.02 ^b
IL-8 (pg/ml)		100.74 \pm 5.53	156.90 \pm 9.75 ^a	120.77 \pm 4.85 ^b	119.03 \pm 4.02 ^b
TNF- α (ng/l)		337.57 \pm 6.74	543.67 \pm 8.03 ^a	372.46 \pm 11.83 ^b	363.39 \pm 3.56 ^b

IL-1 β : Interleukin-1beta; IL-6: interleukin-6; IL-8: interleukin-8; TNF α : Tumor Necrosis Factor Alpha; ^a significant difference between the arsenic and control groups; ^bsignificant differences between arsenic plus Ginger or Omega 3-treated groups and the Arsenic group.

Liver fibrosis and regeneration markers: The arsenic rat group showed a substantial increase (P <0.05) in the percentage area of collagen deposition and VEGF immune expression in both the portal tract and central

vein areas of the liver compared to the control group. In contrast, the groups that received arsenic plus Ginger or Omega 3 showed a significant decrease (P <0.05) in those parameters (Table 4, Figures 2&3).

Table (4): Assessment of Liver fibrosis and regeneration markers in the different studied groups

Parameters		Control	Arsenic	Ars. +Ginger	Ars. +Omega 3
Collagen [μ m] ²	C.V region	1.36 \pm 0.24	3.81 \pm 0.32 ^a	2.18 \pm 0.36 ^b	2.03 \pm 0.32 ^b
	Portal region	1.41 \pm 0.20	3.98 \pm 0.33 ^a	2.54 \pm 0.21 ^b	2.36 \pm 0.21 ^b
VEGF [μ m] ²	C.V region	0.87 \pm 0.19	3.06 \pm 0.38 ^a	1.39 \pm 0.18 ^b	1.38 \pm 0.12 ^b
	Portal region	0.92 \pm 0.11	3.44 \pm 0.44 ^a	1.74 \pm 0.30 ^b	1.52 \pm 0.15 ^b

VEGF: vascular endothelial growth factor; C.V: central vein, ^a significant difference between the arsenic and control groups; ^bsignificant differences between arsenic plus Ginger or Omega 3-treated groups and the Arsenic group.

Microscopic Examination of Liver Tissue: The liver specimen in the control group's central vein and portal tract areas showed standard classic liver architecture. The liver of rats exposed to arsenic exhibited varying degenerative changes in the hepatocytes and excessive widening and congestion of the central vein. The portal region exhibits hepatic artery wall thickening,

inflammatory cellular infiltration, and congestion and dilatation of the portal vein. The arsenic plus Ginger-exposed group and the Arsenic plus omega 3-exposed group showed noticeable improvement in the liver structure and restoration of hepatocytes, liver sinusoids, and portal tract (Fig.1).

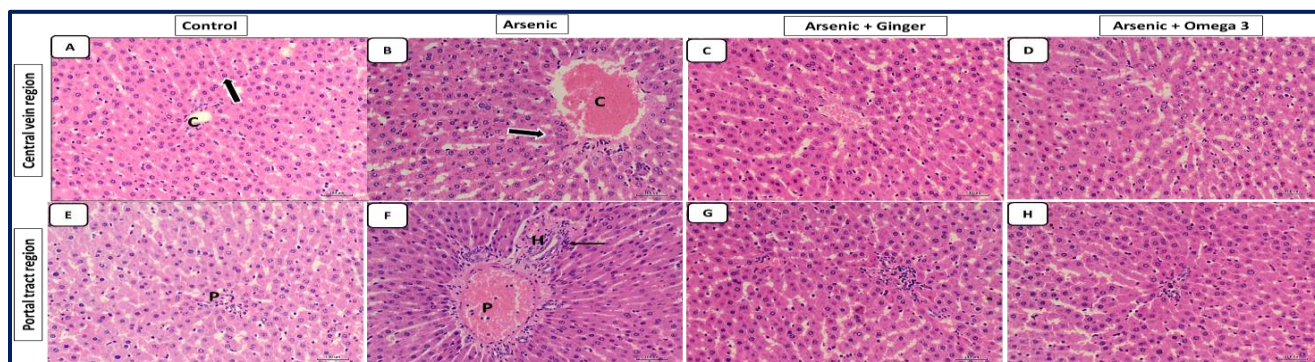


Fig. 1. Photomicrographs of the liver sections stained with H&E in the central vein region {A-D} and the Portal tract region {E-H}, showing {A, E}: standard classic liver architecture of the control group in the form of normal central vein (C) and radiating branching cords of hepatocytes (arrow) and normal portal tract (P). Group exposed to arsenic {B, F} exhibited variable deteriorating changes in the hepatocytes (thick arrow) as well as excessive dilatation and congestion of the central vein (C), the portal region exhibits thickening of the hepatic artery wall (H), excessive inflammatory cellular infiltration (thin arrow), and dilatation and congestion of the portal vein (P). Arsenic plus Ginger-exposed group and Arsenic plus omega 3-exposed group {C, G, D&H} showed a noticeable improvement in liver structure and a reappearance of normal hepatocytes and sinusoids in the portal and central venous areas. (H&E, x 400 scale bar 100 um).

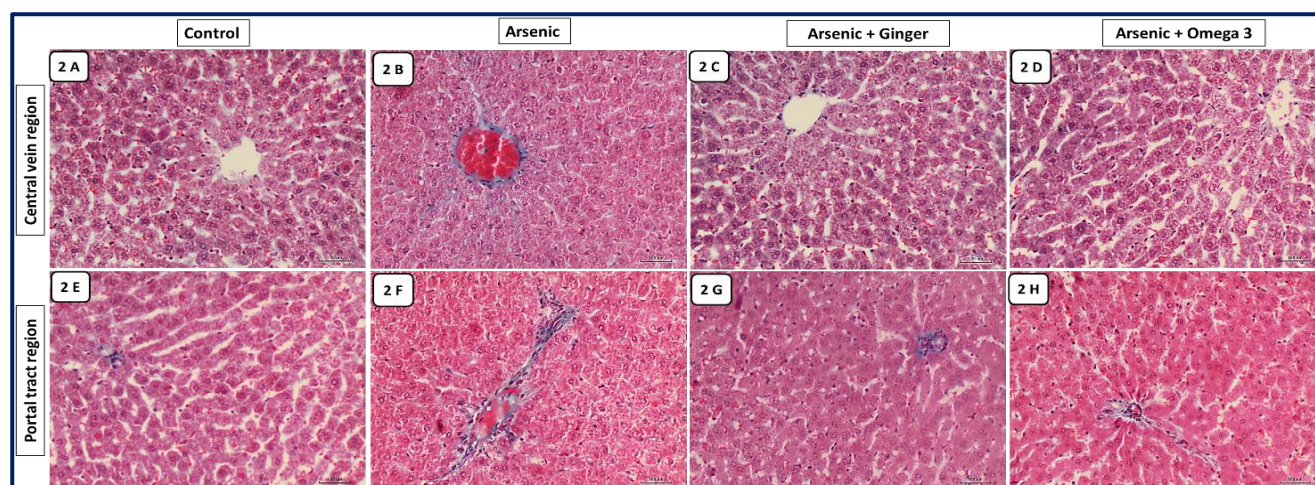


Fig. 2: Photomicrographs of the liver sections showed (A&E): Control group with minimal deposition of collagen fibers (green color) in both the Central vein and portal tract areas; (B&F): Arsenic-treated group revealed extensive deposition of collagen fibers in both the Central vein and portal tract areas. (C-H): Arsenic plus Ginger or omega 3-exposed groups showing minimal deposition of collagen fibers in both the Central vein and portal tract areas (Masson Trichrome stain, x 400 scale bar 100 um).

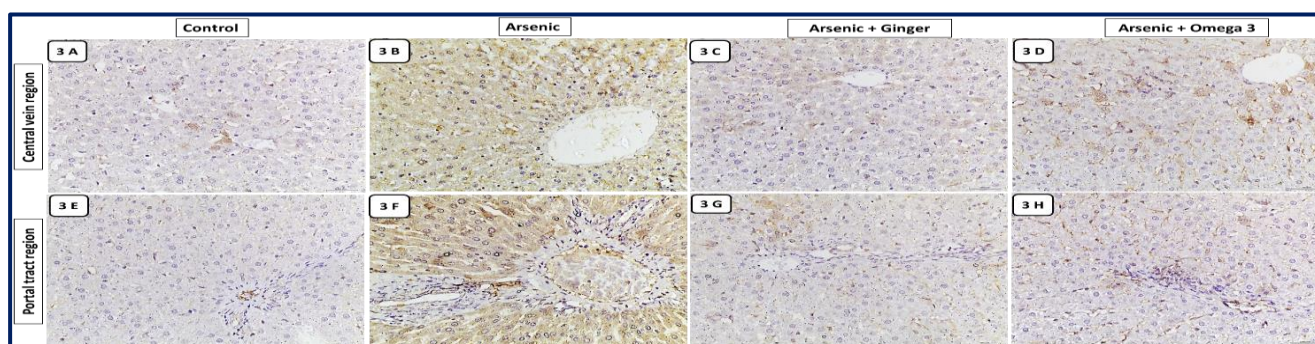


Fig. 3: Photomicrographs of the liver sections showed a marked increase in the immunopositivity of VEGF (dark brown color) deposited in the endothelial lining of the hepatic sinusoids in the arsenic-exposed group when compared to the negative control group (pale stained color). Also, there was a significant decrease in the immunopositivity of VEGF deposition in the endothelial lining of the hepatic sinusoids of rat groups that received either Arsenic + ginger/omega 3 compared to that of the rat group exposed to arsenic only (VEGF immunopositivity, x 400, scale bar 200 pixels).

DISCUSSION

Arsenic is one of the heavy elements that contribute to environmental pollution, which has systemic effects on different human organs [28]. The amount of arsenic in the ecosystem is rising due to the growing environmental dispersion of metals and agricultural pesticides. These heavy metals' pollution poisons wildlife over time [29].

Arsenic accumulates in body tissues through various routes. The liver's role in the metabolism of xenobiotics makes it especially vulnerable [30]. Due to the comparatively longer half-life and delayed body elimination, hepatotoxicity is a severe side effect during liver detoxification [31].

In this study, we assessed the arsenic-induced hepatotoxicity and the possible protective role of supplementation of *Zingiber officinale* or omega-3 to arsenic-exposed rats in drinking water.

Blood samples from rats exposed to drinking water containing arsenic showed increased serum levels of liver enzymes as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), suggesting that arsenic was deteriorating liver functions. This was consistent with earlier research findings [28-29, 32-33], which revealed arsenic toxicity in either liver or kidney functions.

No firm theory explains the precise process driving the increase in these enzymes. Numerous experts have proposed that this phenomenon could stem from cellular impairment or heightened plasma membrane permeability. Furthermore, variables like higher synthesis or lower enzyme degradation could be at play [34-35].

The structural alterations of the hepatic tissue, as demonstrated by the degenerative changes in the hepatocyte and the central vein's excessive dilatation and congestion, further supported the arsenic-induced hepatotoxicity in our investigation. The portal region exhibits thickening in the wall of the hepatic artery, acidophilic exudate, infiltration with inflammatory cells, and congestion and dilatation of the portal vein of the

liver next to arsenic exposure in rats, confirming liver damage. Similar to our results, previous research has demonstrated the structural pathological changes following arsenic delivery in the form of inflammation, necrosis, and apoptosis of the liver after arsenic poisoning in rats or mice, which are indicators of liver injury [29, 34].

Arsenic-induced liver degeneration could be caused by oxidative stress, which may contribute to cellular protein breakdown. Because of arsenic toxicity, hepatocytes may shrink and degenerate, increasing cellular material permeability and leakage and causing sinusoidal enlargement [33-34].

Thus, we assessed the oxidative stress parameters in rat's hepatic tissue after exposure to arsenic in drinking water. We found increased lipid peroxidation (LPO) and nitric oxide (NO) tissue levels. In contrast, the reduced glutathione (GSH) levels, total glutathione (TG), catalase (CAT) activity, and superoxide dismutase (SOD) activity were all decreased in the liver after exposure to arsenic, compared to those levels in the control group. Consistent with this finding, arsenic was found to disturb the internal oxidant and antioxidant balancing mechanisms in the form of decreased levels of SOD, CAT, GPx, and GSH, which are essential in this defense mechanism, while when arsenic accumulates in cells [28, 32].

To summarize, the production of free radicals induced by heavy metals like Arsenic causes oxidative stress, leading to numerous illnesses, including liver damage.

Moreover, several cellular cascades, including inflammatory, apoptotic, and antioxidant pathways, will be activated after the formation of ROS. Activation of pro-inflammatory cytokines such as interleukins IL-1 β , IL-6, IL-8, and tissue necrosis factor alpha (TNF- α) [36-37].

We investigated the serum levels of pro-inflammatory cytokines, and the levels of IL-1 β , IL-6, IL-8, and tissue necrosis factor alpha (TNF- α) were increased in the arsenic-exposed rats compared to the control group. This was in line with the work of other studies,

which revealed that the activation of NF- κ B by oxidative stress can free it from I κ Bs and lead to its translocation to the nucleus, where it controls the gene transcription that causes an increase in pro-inflammatory markers such as TNF α and IL-6 [28, 38].

In this study, arsenic exposure to rats increased collagen deposition in their livers, confirming hepatic fibrosis of liver tissue in both the central and portal tract regions. This was in line with other research findings, which discovered that arsenite exposure in mice resulted in liver damage, as shown by higher fibrosis-related indexes [39-40]. However, it is still unclear how arsenic causes liver fibrosis at the molecular level.

There are several possible causes for hepatic fibrosis caused by arsenic: histone acetylation, oxidative stress, apoptosis, DNA methylation, and epigenetic modifications are all altered by arsenic as well as microRNA-21 (miR-21) mediates abnormal cross-talk between hepatocytes and hepatic stellate cells (HSCs) via the Vascular endothelial growth factor/Hypoxia-inducible factor (HIF)-1 α (HIF-1 α /VEGF) signaling pathway which contributes to hepatic fibrosis induced by arsenic exposure [40-41].

In this study, we assayed the immune expression of VEGF in the liver of arsenic-exposed rats. We found a significant increase in its expression compared to the control group. Previous studies recorded similar findings, which recorded a marked increase in the immune expression of VEGF in the degenerative hepatic areas, which could be a cellular compensatory mechanism [42, 43].

We used several antioxidants, such as (omega-3/ Zingiber officinale) to alleviate the above degenerative hepatic changes. We found that supplementation of either omega-3 or Zingiber officinale with arsenic to rat groups resulted in a significant improvement of liver functions (decrease of ALT, AST, ALP); restoration of most of the structural hepatic changes (restoration of normal hepatocytes, sinusoids, and portal region); reduction of hepatic fibrosis & VEGF immune expression;

improvement of inflammatory parameters (decreased levels of IL-1 β , IL-6, IL-8, and TNF- α); improvement of oxidative stress and inflammatory parameters (reduced levels of LPO, CAT, NO, while, the levels of GSH, TG, and SOD were all increased), those compared to levels in arsenic group.

In agreement with our findings, co-administration of Omega-3 Zingiber officinale to heavy metals-intoxicated animals resulted in significant improvements in the liver tissue and its biochemical parameters compared to heavy metals-intoxicated rats. [25, 44]. Similarly to our results, Omega-3 decreased IL-6 and TNF- α in human patients [45]. That finding suggests both compounds have antioxidative and anti-inflammatory properties, resulting in hepatoprotective effects.

Conclusion: Supplementation of Omega-3 Zingiber officinale to arsenic-intoxicated animals significantly improved the liver structure and function through antioxidative stress, antiapoptotic, and inflammatory effects.

Abbreviations: ALP: alkaline phosphatase, ALT: alanine transaminase, Arsenic: ANOVA: one-way analysis of variance, AST: aspartate aminotransferase, As: Arsenic, BW: body weight, CAT: catalase, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, GSH: reduced glutathione, H&E: hematoxylin and eosin, IL-1 β : interleukins beta, IL-6: interleukins 6, IL-8: interleukins 8, LPO: lipid peroxidation, MCL: maximum permissible concentration level, NO: nitric oxide, SOD: superoxide dismutase, TG: total glutathione, TNF- α : tissue necrosis factor-alpha, VEGF: vascular endothelial growth factor.

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