Significance Assessment of *Amphora coffeaeformis* in Arsenic-Induced Hemato-Biochemical Alterations of African Catfish (*Clarias gariepinus*)

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Heavy metals have different adverse impacts on different life stages of fish species with attempts to use natural antioxidants to counteract their effects. So, the present study investigated the potential protective effects of *Amphora coffeaeformis* extract against arsenic-induced hemato-biochemical alterations in African catfish, *Clarias gariepinus*. The fish exposed to sub-lethal concentrations of arsenic; 19.2 and 38.3 mg/L (1/8 and 1/4 of 96h-LC₅₀ value, 153.17 mg/L) for 15 days. The main effect of arsenic was recorded in some blood parameters such as RBC's count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, and white blood cells. As for biochemical parameters, the main effect of arsenic was significant for alkaline phosphatase, glucose, uric acid, creatinine, albumin, globulin, and albumin/globulin. Also, the residue of arsenic in fish muscles showed significant effects. The majority of these arsenic-induced parameters were improved with dietary supplements of the diatom *A. coffeaeformis*. So, Amphora extract can be used as detoxification factor on fishes induced by arsenic due to its biologically active components providing protections like antioxidant, antiviral, antibacterial, and anti-inflammatory. Besides, they have excellent contents of proteins and carbohydrates which are supposed to enhance the effect of these compounds.

**Keywords**: arsenic, *Clarias*, *Amphora coffeaeformis*, natural product, secondary metabolite, biochemistry, detoxification

**INTRODUCTION**

Different heavy metals including Arsenic, Cadmium, Lead, Silver, and Mercury were found to be toxic to human beings, animals, and fishes with variability in doses and environmental factors (Govind and Madhuri, 2014). Arsenic is one of the most hazardous heavy metals released in the environment as a result of both natural and anthropogenic processes (Garelick et al., 2008). In nature, arsenic exists both in organic and inorganic forms; the inorganic form with trivalent arsenite or pentavalent arsenate are more toxic than organic forms (Oremland and Stolz, 2005). It became evident that the dispersal of arsenic-rich wastes generated by human activities leads to the water pollution and in turn, increased chronic arsenic poisoning of aquatic animals (Rahman et al., 2012).
especially fish juveniles with a reduction in survival and growth of their populations (Erickson et al., 2011). These adverse impacts may lead to the removal of entire fish populations in polluted aquatic ecosystems (Khayatzadeh and Abbasi, 2010). Consequently, accumulation of arsenic at high concentration in fishes may lead to serious health risks for humans, causing cancer and neurological disturbances (Kapaj et al., 2006; Avigliano et al., 2015). To understand the toxicity of arsenic compounds, most studies were performed in mammalian cells (García-Esquinas et al., 2013; Selvaraj et al., 2013). However, studies on arsenic toxicity to aquatic animal species, including fish, are rare. These studies stated that exposure to arsenic led to various hematological and biochemical alterations in fishes (Sayed et al., 2015a; Singh and Srivastava, 2015; Ghaffar et al., 2016). Arsenic was found to promote apoptotic and necrotic mediated cell death in fishes according to variations in arsenic concentration and exposure time (Sayed et al., 2015a). It also leads to DNA fragmentation, alteration in mitochondrial membrane potential and formation of increased reactive oxygen species (Selvaraj et al., 2013).

And because the pollution has already become very widespread, toxicity prevention is not on; and timely mitigation is the possible solution for minimization of the environmental pollutants and their impacts by using plants and algae as the most desirable mitigation technics (Ullah et al., 2015; Mahar et al., 2016; Kumar, 2018). The algae proved to be effective in the hyperaccumulation of heavy metals as well as degradation of xenobiotics (Suresh and Ravishankar, 2004). The microalgae including Amphora, could be a source of a diverse class of bioactive compounds, especially the carotenoids (canthaxanthin and astaxanthin), polyunsaturated fatty acids, sulfated polysaccharides, β-glucans, and vitamins E and C, which are well-documented as bioactive compounds (Lee et al., 2009; El-Sayed et al., 2018). In addition, many researchers are interested in finding any natural antioxidants having safety and effectiveness, which can be substituted for current and commercial synthetic antioxidants (Taghvaei and Jafari, 2015; Kumasani et al., 2017; Glodde et al., 2018). Benthic diatoms as microalgae were considered as natural antioxidants (Lee et al., 2009; El-Sayed et al., 2018). Diatoms such as Amphora possess high metal absorption capacity and high heavy metal fraction rate (Anantharaj et al., 2011) and are able to activate a definite set of biochemical and physiological processes to resist the toxic action of environmental contaminants (Gaur and Rai, 2001). Such characteristics of diatom have encouraged the application of their extract in detoxification for stress-induced fishes as well as protective and antioxidant agents (Sheikhzadeh et al., 2012) in addition to their antibacterial (Choudhury et al., 2005; Manzoor et al., 2013), antiviral (Abdel-Wahab, 2018), and anti-inflammatory factors (Lauritano et al., 2016).

Marine diatoms developed on substrate could be utilized as feed supplements in enhancing the development and survival of aquaculture species (Khatoon et al., 2009). Amphora sp. is regularly used as primary food for larvae of highly valued and praised seafood such as Crassostrea gigas (Pacific oyster), Penaeus semiulatus (green tiger shrimp), Placopesten magellanicus (sea scallop), Crepidula onyx (limpet), and Haliotis sp. (abalone) (Daume et al., 2000; Al-Maslamani et al., 2007; Chiu et al., 2007). Recent results indicated that Amphora supplement is promising as an alternative method to antibiotics for disease prevention in Nile tilapia culture (Ayoub et al., 2019). So, the current investigation is aimed to study the supplementation of Amphora extract and whether it detoxifies and protects C. gariepinus from arsenic exposure.

**MATERIALS AND METHODS**

**Fish Collection**

Ninety healthy fish of the Nile catfish, *Clarias gariepinus* (154.75 ± 146.9 g weight, 30.87 ± 9 cm length) were purchased from a private farm at Assiut, Egypt. Fishes immediately were transported to the Fish Biology and Pollution laboratory at the Department of Zoology, Faculty of Science, Assiut University. The experimental fishes were reared in aerated glass tanks (100 L capacity) and acclimatized for 2 weeks before being used in the experimental study. The experimental fish fed on commercial pellets (3% of fish weight) twice daily. Feces and residual food were aspirated regularly. The water temperature, pH, and dissolved oxygen concentrations were measured daily as 28.8 ± 3°C, 7.6 ± 0.34, and 3.32 ± 4.5 mg/L respectively (light cycle was 12 h light and 12 h dark).

**Amphora coffeaeformis Extract Preparation**

The extract of *Amphora coffeaeformis* was purchased from National Research Center, Cairo, Egypt. *Amphora* extract was sent to the Analytical Chemistry Unit at Assiut University for GC/MS analysis. The results of GC-MS analysis indicated the presence of 51 different compounds (Table 1 and Figure 1). Some of these compounds were identified according to literature (Silva et al., 2014; Salahuddin et al., 2017; El-Sayed et al., 2018) to be biologically active components such as 2,6-Dimethyl-4[3H]-quinazolinone (anticancer heterocyclic compound), Neophytadiene (Terpene; antiviral activity), Phytol (Diterpene; anti-inflammatory activity) and Hexadecanoic acid (fatty acid; antioxidant). The concentrations of total protein (24.25 g/kg) and total carbohydrate (17.92 g/kg) were estimated by using UV-VIS Double Beam Labomed, Inc. PC Scanning Spectrophotometer (Model UVD-2950) while the concentration of total lipid (56.4 g/kg) was estimated by Bligh and Dyer's acidic extraction method (Bligh and Dyer, 1959).

**Experimental Design**

Fish were weighed, measured and classified randomly into nine groups (10 fish/tank) according to two concentrations of arsenic (AS1, AS2), two concentrations of amphora (AM1, AM2) and their combinations (Table 2). Exposure was continuous for 2 weeks and water was changed daily to prevent deterioration of water quality and replenish arsenic levels. Sodium arsenite (Na$_2$HAsO$_4$·7H$_2$O) of 98% purity was purchased from Qualikemes company, India. A stock solution of sodium arsenate was prepared and stored in clean glass bottles.
TABLE 1 | Gas chromatography mass spectrometry activity of Amphora coffeaeformis extract.

| No. | RT (min) | Compound name                                | % of total | Molecular weight |
|-----|---------|-----------------------------------------------|------------|------------------|
| 1   | 4.238   | Benzene (hydrocarbon)                         | 1.857      | 78.047           |
| 2   | 24.615  | Heptadecane (hydrocarbon)                     | 2.393      | 240.282          |
| 3   | 4.279   | Tert-amyl chloride (chlorinated hydrocarbon)  | 4.482      | 106.055          |
| 4   | 4.953   | Bromodichloro-methane (hydrocarbon derivatives) | 0.355      | 161.864          |
| 5   | 12.568  | 1,2-Bis(methoxy)-3-chloropropene (hydrocarbon derivatives) | 0.159      | 138.045          |
| 6   | 23.001  | Hexadecane (hydrocarbon)                      | 0.500      | 226.266          |
| 7   | 40.778  | Eicosane (hydrocarbon)                        | 0.633      | 282.329          |
| 8   | 18.734  | 1,1'-Biphenyl (organic compound)              | 0.251      | 154.078          |
| 9   | 4.419   | Trichloromethane (organic compound)           | 0.178      | 117.914          |
| 10  | 24.819  | 1H-Pyrrolo[1,2-a][1,4]diazepine-1,5(2H)-dione, hexahydro-,(S)-(8CI) (organic compound) | 1.090      | 168.09           |
| 11  | 25.653  | (−)-Loliolide (organic compound)              | 2.067      | 196.11           |
| 12  | 27.85   | Dibutyl phthalate (organic compound)          | 2.536      | 278.152          |
| 13  | 38.546  | Octadecyl 2,2,2-trichloroethyl carboxic acid ester (organic acid) | 1.338      | 444.196          |
| 14  | 30.98   | Benzyl 1-naphthyl ether (organic compound)    | 0.651      | 234.104          |
| 15  | 36.465  | 2-[Tetradecyloxy]-ethanol (organic compound)  | 0.356      | 258.256          |
| 16  | 37.042  | Bis(2-ethylhexyl) phthalate (organic compound) | 0.861      | 390.277          |
| 17  | 37.112  | 1,1,3-Trichloro-2-propanone (ketone)          | 0.416      | 159.925          |
| 18  | 26.51   | 6,10,14-Trimethyl-2-pentadecanone (ketone)    | 1.151      | 288.277          |
| 19  | 5.427   | 3-Hydroxy-3-methyl-2-butanone (ketone)        | 0.438      | 102.068          |
| 20  | 7.654   | 2-Methyl-4-pentene-2-ol (alcohol)             | 0.415      | 100.089          |
| 21  | 26.964  | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (alcohol) | 0.794      | 296.308          |
| 22  | 7.776   | 2-Hexanol (alcohol)                           | 12.927     | 102.104          |
| 23  | 4.547   | 3-Penten-2-ol (alcohol)                       | 1.881      | 86.073           |
| 24  | 9.006   | Iso-valeric acid (fatty acid)                 | 0.797      | 102.068          |
| 25  | 25.495  | Tetradecanoic acid (fatty acid)               | 4.497      | 228.209          |
| 26  | 26.737  | Pentadecanoic acid (fatty acid)               | 1.925      | 242.225          |
| 27  | 30.549  | Oleic acid (fatty acid)                       | 3.138      | 282.256          |
| 28  | 32.933  | 15-Hydroxypentadecanoic acid (fatty acid)     | 0.253      | 258.219          |
| 29  | 38.371  | 9-Octadecenoic acid (fatty acid)              | 0.535      | 504.491          |
| 30  | 36.855  | Oleic acid (fatty acid)                       | 1.318      | 282.256          |
| 31  | 27.906  | Hexadecanoic acid (fatty acid)                | 13.426     | 256.24           |
| 32  | 29.948  | Phytol (diterpene, anti-inflammatory activity) | 1.698      | 296.308          |
| 33  | 26.445  | Neophytadiene (terpene)(antiviral activity)   | 0.771      | 278.297          |
| 34  | 21.812  | Dihydroactinidiolide (terpene)                | 0.314      | 180.115          |
| 35  | 24.423  | 8-Heptadecene (alkene)                       | 2.357      | 238.266          |
| 36  | 26.276  | (2Z)-3-Methyl-2-decene (alkene)               | 1.739      | 154.172          |
| 37  | 36.372  | 1-Nonadecene (alkene)                        | 0.799      | 266.297          |
| 38  | 36.704  | 1-Docosene (alkene)                          | 0.287      | 308.344          |
| 39  | 16.397  | 2-Methoxy-4-aminophenol (phenols)             | 0.231      | 139.063          |
| 40  | 26.638  | 3,5-dimethoxy-Phenol (phenols)                | 0.429      | 194.073          |
| 41  | 25.227  | 4-Methoxy-3-methyl- 6-benzofuranol (antimicrobial compound) | 1.140      | 178.063          |
| 42  | 27.477  | 3-Isobutylhexahydropyrrolo[1,2-a]pyrazine-1,4-dione (antimalarial agent) | 1.796      | 210.137          |
| 43  | 4.804   | 2,6-Dimethyl-4-[3H]-quinazolinoline (heterocyclic compound)(anticancer) | 7.437      | 171.852          |
| 44  | 27.757  | (+−)-15-Hexadecanoamide (antioxidant)         | 12.529     | 254.225          |
| 45  | 44.106  | Cholesterol (sterol)                         | 0.556      | 388.355          |
| 46  | 43.401  | (3, beta,..22)-27-Norgenosta-5,22-dien-3-ol (sterol) | 0.967      | 384.339          |
| 47  | 44.881  | Ergosta-5,22-dien-3-beta.-ol (sterol)         | 0.729      | 398.355          |
| 48  | 48.887  | Stigmasterol (sterol)                        | 0.996      | 412.371          |
| 49  | 30.473  | 8-(2-Octylcyclopropyl)octanal (aldehyde)      | 0.733      | 280.277          |
| 50  | 4.646   | Isoprene hydrochloride (isoprene)             | 0.118      | 117.914          |
| 51  | 27.605  | 14S,20R-Velbanamine (alkaloid)                | 0.372      | 136.125          |
Behavioral Assessments
The behavioral changes were recorded by observing the feeding activity, the fish equilibrium in the water beside the changes in the skin and the fins. The mortality rate was also recorded in the arsenic-exposed fishes.

Hemato-Biochemical Parameters
Two blood samples of the peripheral blood were collected from cardiac puncture. For hematological analysis, samples were freshly collected in small plastic tubes containing heparin solution (0.2 mL/mL blood) as anticoagulant. For biochemical analysis, samples were left in small plastic tubes to coagulate for 15–20 min at 4°C prior then centrifugation for 20 min at 3,000 rpm to separate serum that was used for the analysis. The red (RBCs) and white (WBCs) blood...
cell counts, hematocrit (HCT), and hemoglobin (HB) were estimated by using automated technical analyzer (BCC-3000B-Dirui Company). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formulae mentioned by Dacie and Lewis (1991). Aspartic amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glucose (GL), total protein (TP), albumin (AL), globulin (GLO), creatinine (CR), and uric acid (UA) were determined by kits of HUMAN Company, Germany.

Arsenic Residues
Tissue analyses were done according to Shaw et al. (2012) with minor variations. Muscle samples were weighed (approximately 1.0 g), dried (50°C for 48 h in the oven) and then digested in 5 mL concentrated nitric acid at 50°C in the oven until evaporating the nitric acid and become the mixture 1 mL approximately. The mixture was cooled, diluted to 10 mL using ultrapure deionized water and then filtered. The arsenic residues in the muscles were measured by iCAP 6200 Emission spectrometer in The Central Laboratory, Faculty of Agriculture, Assiut University.

Statistical Analysis
The means, standard errors and ranges of the parameters in concern were estimated. Levene's test of equality of error variance of the parameters was applied with a wide range of variability. So, the homogeneity of variance was assumed for raw data. The pattern of variations in the variables studies was considered on the bases of arsenic and amphora concentrations and their interaction by two-way ANOVA. Moreover, in the absence of interactions, the pattern of variations was recorded by one-way ANOVA in all treatments and control group. The Tukey-HSD test was applied for multiple comparisons. The IBM-SPSS package version 22 (Spss for Windows, 2013) was used at 0.05-level of significance. The relationships between different treatments versus the control group as root group were postulated in dendrograms using R-packages (R Core Team, 2013) and Mesquites package (Maddison and Maddison, 2018) using the hematological and biochemical characters in raw form and ANOVA-based coded form (Rae and Buckley, 2009).

RESULTS

Behavioral Changes
After exposure to arsenic, most of the fishes exhibited loss of equilibrium which was more marked with increased concentration and duration. Reduction in the feeding activity, fins hemorrhage and skin alterations were also recorded in those samples exposed to arsenic. With increased duration, arsenic- exposed fishes showed signs of tiredness and gradually lost positive rheotaxis with excessive secretion of mucus. The mortality rate (60%) was recorded in aquaria with 38.3 mg/L arsenic. The dead fishes exhibited changes and abnormality in eyes, gills, gall bladder, spleen, and liver color. On the other hand, fish groups exposed to the same doses of arsenic in combination with amphora extract did not show such abnormal behavior and did not show any mortality. Moreover, arsenic-free fishes treated with amphora extract were noticed to have a healthy status.

Hemato-Biochemical Alterations
Under treatment conditions of the present work, the uric acid was found to be significantly correlated with ALP (0.453), glucose (0.424), total protein (0.524), and globulin (0.567), whereas glucose was significantly correlated with ALP (0.575). The albumin was significantly correlated with total protein (0.554) and creatinine (0.705). The WBCs was also significantly correlated with RBCs (0.886), HB (0.875), and HCT (0.809).

The hematological and biochemical parameters of the arsenic-induced C. gariepinus versus treatment with Amphora extract are given in Tables 3, 4. The arsenic main effect was significant for RBCs, HCT, MCV, MCH, and WBCs whereas that of amphora was significant for HB and WBCs. There was no significant interaction between arsenic and amphora. As regards the biochemical parameters, the arsenic main effect was significant for ALP, glucose, uric acid, creatinine, albumin, globulin, and albumin/globulin. Also, arsenic residue in muscle showed significant effects by arsenic whereas that of Amphora was significant for ALT, glucose, uric acid, creatinine, LDH, albumin, globulin, and albumin/globulin. The arsenic amphora interaction was significant for ALP, glucose, uric acid, creatinine, LDH, albumin, globulin, albumin/globulin, and arsenic residue in muscles. The main effect of amphora recorded to be significant for glucose, uric acid, creatinine, albumin, globulin, albumin/globulin, and arsenic residue in muscles. The analysis revealed that a higher amphora dose was better than a lower one in counteracting arsenic impact.

Collecting all characters studied, all the treatments were clustered against the control using all the hematological and biochemical parameters relative to their SD units (Figure 2). These treatments are grouped into two main groups. One of these main groups included treatments with amphora only and the arsenic treatment combined with amphora reflecting the validity of amphora extracts as antioxidants. The other main group reflects a partial protective role of Amphora in counteracting the arsenic impacts. Clustering of the treatments based on the ANOVA-based coding of these parameters reflected another pattern of variation (Figure 3).

AS1 treatment was grouped with AS1 AM1 whereas AS2 was clustered with AS2 AM2 in the main group including all treatments with amphora. AS1 AM2 represents a single cluster. Such a pattern of clustering reflects the significance of Amphora protective and antioxidant effects in a collective manner including all characteristics coded on the basis of ANOVA.

DISCUSSION

The toxicity of arsenic highly variable within and between different fish species with respect to factors like age, sex, dose, exposure period and its organic and inorganic forms (Hallauer et al., 2016; Mahurpawar, 2017). These findings are evident with different pollutants and different fish species
TABLE 3 | Values of blood constituent parameters of Clarias gariepinus exposed to arsenic, Amphora and their combinations.

| Treatment/Parameters | Control | As1 | Amphora Am1 | As2+Am1 | As2+Am2 |
|----------------------|---------|-----|------------|--------|--------|
| RBC (millimillions)   | 2.69 ± 0.19a | 2.22 ± 0.02a | 2.25 ± 0.07a | 2.22 ± 0.02a | 2.24 ± 0.05a |
| HB (g/dL)             | 10.07 ± 0.27a | 9.07 ± 0.27a | 8.8 ± 0.40a | 8.78 ± 0.38a | 8.53 ± 0.41a |
| HCT (%)               | 48.13 ± 1.46a | 38.13 ± 0.74a | 32.93 ± 3.08a | 34.13 ± 2.77a | 32.07 ± 2.59a |
| MCV (fL)              | 180.37 ± 4.97b | 160.43 ± 5.08ab | 167 ± 14.47ab | 153.27 ± 14.3ab | 141.27 ± 13.4ab |
| MCH (pg)              | 24.17 ± 12.6a | 19.5 ± 9.29ab | 25.53 ± 1.23a | 24.37 ± 26.75b | 25.37 ± 27.58b |
| WBC (Thousands/mm³)  | 173.90 ± 18.35a | 130.45 ± 16.49ab | 128.5 ± 9.21ab | 237.90 ± 18.35a | 253.7 ± 26.75b |

The data are presented as Means ± SE (minimum–maximum). Different letters indicate significant difference at p < 0.05. As1 (19.2 mg/L Arsenic), As2 (38.3 mg/L Arsenic), Am1 (7% Amphora), Am2 (10% Amphora).

The excessive secretion of mucus which was observed especially at high doses of arsenic was probably due to skin arsenic-induced irritation and hence to protect the skin. Similar findings were recorded by Singh and Banerjee (2008). Impairments of the nervous system such as reflected in the form of swimming with the sides twisted 90 degrees and loss of equilibrium were also recorded in the present study under the stress of arsenic. Such impairments were postulated by Patro (2006) and Dwivedi and Trivedi (2015). Supplementation of Amphora to the arsenic-treated groups of C. gariepinus as well as untreated groups counteracted and improved the above behavioral changes to a great extent. These findings are suggested to be due to the multiple positive role of bioactive compounds of A. coffeaeformis working as antioxidant, antibacterial, antiviral, anti-fungal, and anti-inflammatory (Rajput and Mishra, 2012; Salahuddin et al., 2017; El-Sayed et al., 2018).

The alterations in RBC's, HB, and HCT observed in the present study may have resulted from the disorders in hematopoietic processes due to sodium arsenate toxicity. Heath (1987) and Abo-Hegab et al. (1993) interpreted the stress-induced decrease in the hemoglobin and hematocrit values in terms of heme dilution of blood and elimination of RBCs as well as disequilibrium of the osmotic pressure inside and outside the blood cell. Similar results were observed in fish intoxicated with arsenic, pesticides, SDS, lead, and silver nanoparticles (Mekkawy et al., 2013, 2019; Amsath, 2017; Sayed et al., 2017; Sayed and Authman, 2018). Arsenic-induced changes in MCV, MCH, and WBCs of current species were evident. Some other studies reported similar results with fluctuations in some fish such as Clarias batrachus, and Catla catla (Lavanya et al., 2011; Kumar and Banerjee, 2016).

Low white blood cells of C. gariepinus were observed in the present study after arsenic exposure. According to Kotsanis et al. (2000) and Datta et al. (2009), the decrease in white blood cell counts during acute and sub-lethal treatment by arsenic may be attributed to the damage of the kidney, which is the primary site of hematopoiesis and/or due to inhibition of white blood cell maturation due to arsenic stress.

The liver is the major organ involved in the regulation of metabolic functions and most of the biotransformation of inorganic arsenic takes place in the liver (Kumar and Banerjee, 2016; Kumari et al., 2017). So, analysis of serum AST and ALT were widely used to demonstrate arsenic induced-hepatotoxicity (Dorcas and Solomon, 2014). The alterations of AST and ALT reported in the present study may be due to the rapid death...
## TABLE 4 | Values of blood constituent parameters and arsenic residue in the muscle of *Clarias gariepinus* exposed to arsenic, amphora and their combinations.

| Treatments/Parameters | Control | As1 | As2 | Am1 | Am2 | As1+Am1 | As1+Am2 | As2+Am1 | As2+Am2 |
|-----------------------|---------|-----|-----|-----|-----|---------|---------|---------|---------|
| **AST (U/mL)**        | 226.5 ± 35.82 a | 284.4 ± 45.17 ab | 384.5 ± 53.61 b | 269.5 ± 10.69 ab | 256.9 ± 18.93 a | 275.3 ± 6.81 ab | 242.3 ± 16.04 a | 247.4 ± 24.63 a | 239.3 ± 12.6 a |
| (155.3-269.1)         | (211–366.7) | (323–439.6) | (250.2–286.7) | (222.8–288.2) | (267.8–288.9) | (210.6–262.4) | (205.3–290.6) | (223.5–264.2) |
| **ALT (U/mL)**        | 34.63 ± 3.36 a | 33.57 ± 7.18 a | 40.03 ± 5.32 a | 24.34 ± 2.01 a | 41.18 ± 1.26 a | 25.26 ± 2.26 a | 31.71 ± 2.46 a | 28.18 ± 4.31 a | 27.75 ± 2.78 a |
| (28.01–38.97)         | (21.3–46.16) | (29.63–47.18) | (22.27–28.36) | (29.61–43.66) | (21.12–28.92) | (27.77–36.22) | (20.1–34.81) | (22.35–31.59) |
| **ALP (U/L)**         | 44.28 ± 8.72 a | 17.52 ± 6.82 ab | 3.19 ± 1.31 b | 30.09 ± 3.96 ab | 23.68 ± 1.14 ab | 19.33 ± 0.87 ab | 15.94 ± 4.6 b | 27.01 ± 8.01 b |
| (30.7–60.54)          | (5.23–28.77) | (12.7–5.69) | (23.47–37.17) | (21.68–25.62) | (12.36–15.36) | (12.69–21.28) | (7.29–22.94) | (16.33–42.69) |
| **Glucose (mg/dL)**   | 71 ± 1.53 a   | 54.67 ± 2.67 ab | 54 ± 3.06 abc | 62.33 ± 6.84 ab | 54 ± 3.06 abc | 45.67 ± 6.89 ab | 38.33 ± 3.18 bc | 27 ± 4.51 d | 49.33 ± 1.2 b |
| (69–74)               | (52–60)       | (55–76)       | (50–60)       | (36–59)       | (33–44)       | (22–36)       | (47–51)       | (51–35)       |
| **Uric acid (mg/dL)** | 0.88 ± 0.06 a | 0.41 ± 0.06 b | 0.43 ± 0.04 b | 0.33 ± 0.01 b | 0.49 ± 0.02 b | 0.46 ± 0.05 b | 0.4 ± 0.01 b | 0.46 ± 0.02 b | 0.36 ± 0.02 b |
| (0.76–0.95)           | (0.33–0.52)   | (0.36–0.51)   | (0.31–0.35)   | (0.48–0.5)    | (0.36–0.52)   | (0.38–0.42)   | (0.38–0.49)   | (0.33–0.39)   |
| **Creatinine (mg/dL)**| 0.51 ± 0.04 a | 0.71 ± 0.04 b | 0.38 ± 0.02 ac| 0.27 ± 0.06 c | 0.35 ± 0.01 ac| 0.53 ± 0.02 ab| 0.4 ± 0.03 ac| 0.38 ± 0.03 ac| 0.38 ± 0.03 ac|
| (0.45–0.59)           | (0.65–0.8)    | (0.34–0.41)   | (0.16–0.33)   | (0.32–0.37)   | (0.5–0.56)    | (0.42–0.43)   | (0.32–0.57)   | (0.32–0.42)   |
| **LDH (U/L)**         | 2966.33 ± 483.88 ab | 3618.67 ± 182.47 ab | 4056.67 ± 430.35 b | 2655.33 ± 870.37 ab | 2546 ± 240.22 ab | 2653.33 ± 654.53 ab | 3459.67 ± 363.13 ab | 3735.67 ± 112.17 ab | 1601.33 ± 357.3 a |
| (2055–3704)           | (3306–3938)   | (3269–4751)   | (1417–4334)   | (2108–2926)   | (1240–3650)   | (2970–4169)   | (3622–3960)   | (1100–2293)   |
| **Albumin (mg/dL)**   | 1.27 ± 0.13 ad | 1.69 ± 0.05 b | 0.96 ± 0.03 ac| 1.02 ± 0.09 ac| 1.1 ± 0.12 ac| 1.53 ± 0.02 bd| 1.21 ± 0.07 ad| 1.25 ± 0.06 c| 0.85 ± 0.06 c |
| (1.04–1.47)           | (1.6–1.75)    | (0.92–1.01)   | (0.85–1.16)   | (0.87–1.25)   | (1.5–1.58)    | (1.1–1.33)    | (1.25–1.26)   | (0.83–0.97)   |
| **Globulin (mg/dL)**  | 2.77 ± 0.17 a | 2.07 ± 0.06 b | 1.78 ± 0.17 cd| 1.55 ± 0.03 d | 2.47 ± 0.11 ab| 1.51 ± 0.13 d | 2.28 ± 0.21 ab| 2.4 ± 0.02 ab | 1.96 ± 0.14 ab |
| (2.59–3.1)            | (2–2.19)      | (1.45–1.95)   | (1.51–1.61)   | (2.34–2.69)   | (1.27–1.71)   | (1.95–2.44)   | (2.89–3.44)   | (1.74–2.22)   |
| **A/G**               | 0.46 ± 0.03 a | 0.86 ± 0.03 b | 0.54 ± 0.04 a | 0.66 ± 0.05 a | 0.45 ± 0.06 a | 1.02 ± 0.09 b | 0.53 ± 0.03 a | 0.52 ± 0.01 a | 0.45 ± 0.01 a |
| (0.4–0.5)             | (0.8–0.9)     | (0.46–0.63)   | (0.56–0.72)   | (0.32–0.52)   | (0.88–1.18)   | (0.48–0.59)   | (0.51–0.53)   | (0.43–0.47)   |
| **Arsenic in muscle** | 0.1 ± 0.01 ab | 0.02 ± 0.01 ab| 0.052 ± 0.0 c | 0 ± 0 a       | 0.01 ± 0.0 a  | 0.03 ± 0.01 d | 0.04 ± 0.01 cd| 0.02 ± 0.01 bd| 0.03 ± 0.01 bd|
| (0–0.01)             | (0.017–0.031) | (0.051–0.052) | (0–0.01)      | (0–0.01)      | (0.02–0.04)   | (0.03–0.05)   | (0.02–0.03)   | (0.02–0.03)   |

The data are presented as Means ± SE (minimum–maximum). Different letters indicate significant difference at p < 0.05. As1 (19.2 mg/L Arsenic), As2 (38.3 mg/L Arsenic), Am1 (7% Amphora), Am2 (10% Amphora).
of numerous liver cells (extensive hepatic necrosis) which were observed in association with liver inflammation, injury, stress, and disease (Dorcas and Solomon, 2014). Similar observations were done in *C. gariepinus* and other fish species exposed to arsenic (Roy and Bhattacharya, 2006; Abdel-Hameid, 2009), 4-nonylphenol (Sayed and Soliman, 2018), cadmium (Mekkawy et al., 2011b), and atrazine (Mekkawy et al., 2013).

Different authors referred to ALP alteration in *C. gariepinus* and different fish species under stress and environmental pollution (Ezenwaji et al., 2013; Sayed and Soliman, 2017). Garima and Himanshu (2015) postulated a decrease in ALP of *C. batrachus* after exposure to arsenic trioxide due to the decrease of food intake in arsenic-exposed fishes.

Different studies reported the significance of LDH alteration under stress in fishes (Mekkawy et al., 2010; Sayed and Soliman, 2018). Moreover, it was suggested that the emergency needs of increased energy demands can be met by the lactate production for gluconeogenesis in the liver to increase LDH activity after stress (Rani et al., 2017). Altered levels of LDH were recorded in the present work and confirmed by other studies on the same species (Sayed et al., 2011; Akinrotimi et al., 2018).

In the present study, reduced glucose level was observed in arsenic-exposed fish. Similar observations have been reported by Lavanya et al. (2011) after exposure of fingerling *C. catla* to arsenic trioxide and by Garima and Himanshu (2015) after exposure of *C. batrachus* to the same pollutant. Acute treatment by arsenic caused hypoxia which leads to an excess utilization of stored carbohydrates, since the glucose level decreases at such stress conditions (Garima and Himanshu, 2015; Kumari et al., 2017).

The arsenic-exposed fishes exhibited alterations in serum protein in the present study as well as in other studies (Garima and Himanshu, 2015; Singh and Srivastava, 2015). Such alterations are interpreted to be due to impaired protein synthesis produced by reactive oxygen species and free radicals (Lavanya et al., 2011; Carlson et al., 2013).

Urea and creatinine have been used as important indicators of renal health in toxicant-induced fish using a variety of both *in vivo* and *in vitro* methods (Davis and Beandt, 1994; Ajeniyi and Solomon, 2014). In the present study, the arsenic-exposed fish exhibited an increase in the creatinine at low arsenic concentrations which may be due to oxidative damage (Prusty et al., 2011), and a decrease under the high arsenic concentration which severely disturbs the metabolic processes in the kidney (Rana et al., 2018). A similar increase in creatinine level was observed by Kumari (2015) in *Oryctolagus cuniculus* exposed to arsenic. However, a decrease in creatinine level was observed by Ogamba et al. (2011) and Inyang et al. (2018) in *C. gariepinus* exposed to paraquat dichloride and lindane, respectively. Uric acid was found to decrease under arsenic stress on *C. gariepinus* of the present work and that of Ogamba et al. (2011). Different other studies revealed an increase in uric acid under arsenic stress.
A high level of arsenic accumulation in the muscles was observed in the present study. These findings are according to the results of Tyokumbur et al. (2014) who reported that the range of arsenic levels in the organs of *C. gariepinus* was highest in the muscles. The studies of Kim and Kang (2015) demonstrated that arsenic exposure can induce considerable arsenic accumulation in major tissues of different fish species (Al Sayegh Petkovšek et al., 2012; Gao et al., 2018).

According to Kaparapu (2018), microalgae like *Amphora*, *Chlorella*, and *Isochrysis* species are utilized as live feed for all growth stages of bivalve molluscs, for larval juvenile stages of abalone, crustaceans and some fish species, and for zooplankton used in aquaculture food webs. However, little is known regarding the role of *Amphora* extract in ameliorating the toxic damages induced by heavy metals in fishes in spite of its bioactive compounds recorded in the present work by GC-MS analysis. These compounds show effect as antioxidant, antibacterial, antiviral, anti-fungal, and anti-inflammatory (Salahuddin et al., 2017; El-Sayed et al., 2018; Munir et al., 2018).

In the present study, amphora bioactive compounds improved most of the plasma biochemical parameters for arsenic-induced fish reflecting, in turn, cytotoxic effects. So, the modulation effect of *Amphora* observed in the current study was in agreement with that of Ayoub et al. (2019) who utilized *Amphora* supplement in improvement of the lysozyme, serum protein and increased disease resistance of Nile Tilapia to *Aeromonas hydrophila* infection and El-Sayed et al. (2018) who utilized *Amphora* supplement as detoxification factor against paracetamol stress in liver tissue of rats. Moreover, the existing concentration of carbohydrates and proteins recorded in the present study may contribute to enhance the antioxidative activities of *Amphora* species as postulated by Rupérez et al. (2002) studying other diatoms.

In the present work, the main effects of arsenic and amphora and their interactions were represented by cluster analysis rooted by the control in the concept of multivariate sense for whole individuals since the treated groups may represent different populations in the environment. Although, the results of *Amphora* against arsenic toxicity showed positive effects, more studies are required to indicate the mechanisms of those effects.

## CONCLUSION

The supplementation of amphora extracts can be used as detoxification and protective factors for *C. gariepinus* induced by arsenic due to the biologically active components of *Amphora* with antioxidant, antiviral, antibacterial, and anti-inflammatory characteristics, besides the abundant contents of proteins and carbohydrates which enhance these components.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

## ETHICS STATEMENT

The animal study was reviewed and approved by the Faculty of Science, Assiut University.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.
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Amphora.
Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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