Assiut Univ. J. of Zoology **Printed ISSN 1687-4935**
49(2), pp. 35-56 (2020)

AMPHORA COFFEAEFORMIS MODULATE THE HISTOPATHOLOGICAL AND HISTOCHEMICAL DISRUPTION IN KIDNEY AND GILLS OF *CLARIAS GARIEPINUS* INTOXICATED BY ARSENIC

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Received: 17/9/2020 **Accepted:** 25/11/2020 **Available Online:** 1/12/2020

The arrival of pollutants such as heavy metals to the aquatic medium is known to affect such a domain and on its living life forms with endeavors to utilize the antioxidant agents to balance their effects. The present investigation explored the potential defensive impacts of *Amphora coffeaeformis* against arsenic-induced histopathology and histochemical adjustments in African catfish (*Clarias gariepinus)*. Fish were exposed to sub-lethal doses of arsenic as 19.2 and 38.3 mg/l for 15 days. Kidneys of arsenic-exposed fish displayed tubular edema and necrosis, hematopoietic degeneration, melanomacrophage aggregates, Bowman's capsule edema and glomerulus shrinkage while gills of arsenic-exposed fish revealed lamellar rupture, lamellar aneurysm, edema, epithelial hypertrophy and mucous cell hyperplasia. PAS response revealed consumption in the carbohydrate materials in the kidney and gills in the arsenic group in contrast with control. The changes induced after arsenic-exposure was improved in fish fed amphora because of their naturally active components that can be utilized as antioxidant, antiviral, antibacterial, and anti-inflammatory for detoxification process.

Keywords: Arsenic; *Amphora;* gills; kidney; PAS response.

INTRODUCTION

As of late, heavy metal contamination has expanded because of mining, natural catastrophic events in addition to human and manufacturing actions [1]. Consistent introduction of different heavy metals, for example, cadmium, arsenic, zinc, copper, nickel and lead have a wide scope of pernicious impacts on living creatures [2, 3]. Albeit many heavy metals are needed for

biochemical alteration at low doses, their bioaccumulation in the crucial organs of the aquatic animals would unfavorably influence the physiological capacities, particularly in fishes [4]. These outcomes are apparent with diverse fish species including *Clarias gariepinus* [5, 6] and *Oreochromis niloticus* [7, 8] that exposed to different toxicants.

Arsenic (As) metal has a Prominence in the worldwide medical issue influencing a large number of individuals. Its pollution brought about by regular topographical sources draining into springs, tainting drinking water and may likewise from mining and other industrial procedures [9]. The harmful effects of the arsenic was found to display intra-and interspecific variable degrees on the bases of various factors including sex, age, portion, exposure time, and its organic and inorganic nature [10, 11].

Arsenic from debased water can enter the body of a fish and concentrate in various organs [12]. According to Zhang *et al*. [13], the process of arsenic biotransformation that takes place within the cell produces a range of products as metabolic processes, some of them come out with the outputs and others remain inside the cell which leads to the formation of free radicals that begin to deteriorate the vital organs. That's why several literatures utilized the histopathological and histochemical adjustments in various tissues as significant tools for fish toxicology as Mekkawy *et al*. [14] and Sayed and Younes [15]. One of the extraordinary points of interest of utilizing histological biomarkers in the fish is the examination of the particular objective organs as gills and kidneys that are in charge of breath, osmoregulation and excretion [16]. Therefore, the study of these organs after their exposure to pollutants received great attention from many scientists as Derakhsh *et al*. [17] and Mahmoud *et al*.[18].

As of late, there has been expanding enthusiasm for utilizing bioremediation as the most alluring innovation utilizing plants to remove

environmental toxins and their impacts [19-21]. The algae demonstrated successful in the inordinate amassing of heavy metals just as in the decay of xenobiotics [22]. Microscopic algae, including *Amphora,* can be a wellspring of an assortment of bioactive substances, particularly carotenoids, fats, carbohydrates, and vitamins, which are all around recorded as naturally bioactive compounds [23, 24]. What's more, numerous scientists are keen on finding any successful normal antioxidants, which can be supplanted by manufactured antioxidants [25, 26].

Benthic diatoms for instance of microalgae have turned out to be great wellsprings of natural antioxidants [27, 28]. Diatoms, for example, *Amphora* have a high assimilation limit of metals and high increase rates [29] and are fit for enacting a particular arrangement of physiological procedures to oppose the dangerous impacts of ecological poisons [30]. These properties of diatom empowered the utilization of its extract in the detoxification of stress-initiated fish just as defensive agents and antioxidants [31], antibacterial [32, 33], antiviral [34], and anti-inflammatory agents [35]. Hence, the present research plans to study changes in the histological and histochemical modifications of the gills and kidney of *Clarias gariepinus* intoxicated by arsenic in the light of *Amphora* extract supplements as detoxification and defensive factors.

1- MATERIALS AND METHODS

2.1. Fish, chemicals, and Amphora

Healthy Nile catfish; *Clarias gariepinus* (154.75±146.9 g weight and 30.87± 9 cm length) were transported to the Laboratory of Fish Biology and Pollution for laboratory adaptation. The trial fish were raised in pneumatic glass tanks (100 liters) and adjusted for about fourteen days before being utilized in the test study. Exploratory fish were fed on commercial pellets (3% by weight of fish) twice every day. Stool and remaining food were daily

excluded. Water temperature, dissolved oxygen and pH concentration were estimated day by day as 28.8 ± 3 ° C, 3.32 ± 4.5 mg /l, and 7.6 ± 0.34 respectively (12 hrs: 12 hrs as light: dark).

98 % pure Sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) was obtained from Qualikemes company, India while the extract of *Amphora coffeaeformis* was obtained from NRC, Egypt. Full characterization of the Amphora extract was done previously by Mekkawy *et al.* [36] .

2.2. Experimental design

Fishes were arranged into 9 gatherings (10 fish/ tank), two groups exposed to 19.2 and 38.3 mg/l arsenic (AS1, AS2) (1/8 and 1/4 of 96h-LC_{50} value, 153.17mg/l) according to Abdel-Hameid [37] and other two groups exposed to 7 and 10 % amphora (AM1, AM2), and other groups exposed to arsenic and amphora combinations for two weeks [36]. During exposure water was changed every day to counteract disintegration of water quality and renewal of arsenic levels.

2.3. Histological and histochemical preparation

For microscopic preparations, 3 surviving fish from each group were sacrificed. Small pieces of kidneys and gills were fixed in 10% formalin. Fixed tissues were paraffin embed, sectioned at a thickness of 5µ, and stained with Harris' hematoxylin and eosin stain (HE) as displayed by Bancroft and Steven [38]. As per Mc Manus [39], the polysaccharide exhibit was demonstrated utilizing periodic acid Schiff's technique (PAS). Alcian blue and periodic acid-Schiff method (AB&PAS) was visualized by Mowry [40] and displayed by the appearance of blue color of the acid mucin, purple color of the neutral mucin and a combination of the two colors for carbohydrate.

3. RESULTS

3.1. Kidney

3.1.1. Control fish and fish fed with Amphora

The kidney of control fish *C. gariepinus* showed normal structure without observed alterations in Bowman's capsule, glomerulus, and Bowman's space (Fig. 1a). Examination of kidney sections of fish *C. gariepinus* administered Amphora displayed typical renal corpuscle, renal tubules and haemopoitic tissue like those of control (Fig. 1b). The marked PAS response was distinguished in the glomerular structures and the renal tubules of the control fish. The glomeruli, basement membranes, as well as the brush borders were fundamentally shaded with the PAS response (Fig. 2a). After feeding fish with amphora, histochemical examination revealed a lot of carbohydrates as a control. This showed up in a profoundly stained glomeruli, brush borders and basement membranes (Fig. 2b).

3.1.2. Fish exposed to arsenic

Examination of kidney sections of fish exposed to 19.2 mg/l arsenic revealed shrinkage in the glomerulus with remarkable observed Bowman's space and degeneration in the haemopoietic tissue. Besides, necrosis was seen in some renal tubules and melanomacrophages were likewise observed (Fig. 1c). In fish exposed to 38.3 mg/l arsenic, kidney sections displayed noteworthy proliferation in the haemopoietic tissue and glomerular deformity (Fig. 1d). What's more, renal tissue displayed extreme harm to renal tubules that demonstrated division with desquamation and exfoliation of cells in the lumen. In addition, edema was seen in renal tubules with reduced lumen. Melanomacrophages have been seen additionally (Fig. 1d). In fish exposed to 19.2 or 38.3 mg/l arsenic, PAS response revealed a consumption of carbohydrates saw in different degree of faint coloration of glomeruli, brush borders and basement membranes (Fig. 2c, d).

3.1.3. Fish fed Amphora and exposed to 19.2 mg/l arsenic

Examination of kidney sections of fish administered 7% amphora in combination with 19.2 mg/l arsenic exposure showed moderate improvement in the renal tissue like control. The renal tubules and glomeruli displayed typical structures (Fig. 1e). In fish fed on 10% amphora in addition to 19.2 mg/l arsenic exposure, kidney sections displayed improvement in glomeruli and some renal tubules while the haemopoietic tissue showed degeneration (Fig. 1f). After fish administration to 7% or 10% amphora in addition to 19.2 mg/l arsenic exposure, the PAS response revealed enormous measure of carbohydrates saw in the glomerulus, basement membrane and haemopoietic tissue (Fig. 2e, f).

3.1.4. Fish fed Amphora and exposed to 38.3 mg/l arsenic

Examination of kidney sections of fish administered 7% amphora in addition to 38.3 mg/l arsenic exposure showed moderate improvement. The renal tissue showed up better with typical glomeruli and ordinary haemopoietic tissues while the renal tubules still display some dissociation (Fig. 1g). After fish administered 10% amphora in addition to 38.3 mg/l arsenic exposure, the renal tubules and glomeruli revealed high improvement in their structure (Fig. 1h). In fish fed on 7% amphora in addition to 38.3 mg/l arsenic exposure, PAS response revealed mild measure of carbohydrates saw in the glomeruli (Fig.2g) while in fish fed on 10% amphora in addition to 38.3 mg/l arsenic exposure, the PAS response revealed a lot of carbohydrates distributed around the kidney tissue (Fig. 2h).

3.2. Gills

3.2.1. Control fish and fish fed with Amphora

The light microscopic examination of the control gills shows the organization of the lamellae. The primary lamellae (PL) are multilayered with its interior part irrigated by blood sinusoids. The respiratory coating of

the secondary lamellae (SL) comprises of a single layer of flattened epithelial cells situated on the pillar cell - blood capillaries (PC-BC) framework which structures the fundamental vascular part of the gills. A surface layer covering the past framework comprises of cubic epithelial cells (Fig. 3a). Examination of gill sections of fish fed on 7% or 10% amphora displayed ordinary gill tissue with primary and secondary lamellae organized like control (Fig. 3b). AB&PAS stain showed ordinary dissemination of the polysaccharides in the gill tissues of the control fish. Such dissemination was detected in the mucous cells (MCs) and PC-BC framework of the secondary lamellae (Fig. 4a). In fish fed on amphora, AB&PAS stain revealed an incredible staining of PC-BC framework with ordinary number and size of MCs (Fig.4b).

3.2.2. Fish exposed to arsenic

In fish exposed to 19.2 mg/l arsenic, the gill histological modifications incorporate hypertrophy of the epithelium and widening of the peripheral channel of secondary lamellae lead to laceration in lamellae. Likewise breakdown in the PC-BC framework's arrangement was observed (Fig. 3c). Fish exposed to 38.3 mg/l arsenic showed serious engorged blood with notable edema in the secondary lamellae. Breakdown in the PC-BC framework's arrangement was observed while other lamellae displayed stamped necrosis (Fig 3d). Broad degeneration in the primary lamellae was observed. In addition, the pillar cells showed up much decreased in size due to laceration of the epithelium. Likewise stamped aneurysm was detected (Fig. 3d). In arsenic-exposed fish, AB&PAS stain revealed a pale color of PC-BC arrangement of the secondary lamellae. Additionally the mucous cells expanded in their size and number (Fig. 4c, d).

3.2.3. Fish fed Amphora and exposed to 19.2 mg/l arsenic

In fish fed on 7% amphora in addition to 19.2 mg/l arsenic exposure, no notable improvement in the gill lamellae was observed. A bit of

hypertrophy and degeneration in the primary lamellae still present (Fig. 3e). The gill tissue revealed moderate repair compared with arsenic treatment after fish fed on 10% amphora in addition to 19.2 mg/l arsenic exposure. Hyperplasia in the primary lamellae was observed. Additionally the PC-BC framework showed little crumples (Fig. 3f). After feeding on 7% or 10% amphora in addition to 19.2 mg/l arsenic exposure, histochemical stains revealed ordinary number and size of MCs and featured color of PC-BC framework (Fig. 4e, f).

3.2.4. Fish fed Amphora and exposed to 38.3 mg/l arsenic

The gill tissue showed unmistakable repair after feeding on 7% amphora in combination with 38.3 mg/l arsenic exposure compared with arsenic treatment. The ladder- like arrangement of the PC-BC framework seemed ordinary and no hypertrophy in the epithelial cells (Fig.3g). After feeding on 10% amphora and 38.3 mg/l arsenic exposure, the gill tissue displayed high improvement when compared with arsenic treatment. The secondary lamellae turned out to be longer with various blood capillaries. The arrangement of the PC-BC framework seemed typical. No degeneration or aneurysm was observed (Fig. 3h). After feeding on 7% amphora in addition to 38.3 mg/l arsenic exposure, AB&PAS stain revealed ordinary number and size of the mucous cells and manifest color of the basement membrane was watched (Fig.4g) while after feeding on 10% amphora in addition to 38.3 mg/l arsenic exposure, AB&PAS stain revealed a pale color in the basement membrane covering the (PC-BC) framework (Fig.4h).

2- DISCUSSION

Fish kidney gets much the biggest extent of post-branchial blood, and accordingly renal injuries may be relied upon to be great pointers of ecological contamination [41]. Shrinkage in the glomerulus with checked enormous Bowman's space, haemopoietic tissue degeneration, and necrosis in

some renal tubules were seen in the present investigation and furthermore seen by Ibrahem [42] on *C. gariepinus* intoxicated by phenol; Xing *et al*. [43] on common carp intoxicated by atrazine and chlorpyrifos; Mekkawy *et al.* [14] on *O. niloticus* exposed to cadmium; Sayed and Younes [15] on *C. gariepinus* exposed to silver nanoparticles; Mirghaed *et al*. [44] on *Cyprinus carpio* exposed to pesticides. Such histopathological alterations were relevant with the existence of lethal substances in the filtrate from the glomerulus according to Silva and Martinez [45]. This interpretation is compatible with the present results shown on the kidney tissue after arsenic-intoxication.

The current data showed positive PAS materials which diminished in the kidney tissues of arsenic-intoxicated fish. This finding is predictable with different examinations, which displayed consumption in the carbohydrate materials of the kidney of *C. gariepinus* intoxicated by lead [46], *Labeo rohita* exposed to arsenic [47], *O. niloticus* exposed to cadmium [14].

The gills, which take part in numerous significant functions in fish, for example, excretion, breath, and osmoregulation, is in direct contact with the changes in the nature of the water and therefore viewed as the primary objective of the contaminants [48, 49]. The variations like hypertrophy of the epithelial cells, other than incomplete crack or combination of some secondary lamellae which displayed in the present examination are instances of defense process. Such variations were manifest in gills of *Acanthopagrus latus* [50] and *C. carpio* [51] exposed to heavy metals.

Serious massed blood in the gills with unmistakable oedema was observed in the present examination. Comparable outcomes were seen in the gill tissue of [52] and *C. gariepinus* exposed to fenthion pesticide [53]. It was viewed as that total edematous partition of the respiratory epithelium of primary and secondary lamellae will bring about lamella epithelial cell necrosis in this manner prompting respiratory and osmoregulatory troubles

[14, 54]. Aneurysm was manifested in the present investigation. It is associated with the laceration of the pillar cells according to Martinez *et al*. [55] because of a greater progression of blood or even in view of the immediate impacts of contaminants on these cells.

In the present investigation, the epithelial layer covering the PC-BC framework of the secondary lamellae is collapsed and falls apart into small fragments as part of deterioration process. Comparative outcomes were seen in the gill tissue of *Heteropneustes fossilis* [56] and *Cirrhinus mrigala* [52] exposed to different toxicants. The goblet mucous cells displayed changes in their thickness and staining conduct in the present examination. Comparative outcomes in such cells were notable by Parashar and Banerjee [56] on *H. fossilis* exposed to lead nitrate and Mekkawy *et al.* [14] on *O. niloticus* intoxicated with cadmium. According to Moron *et al*.[57], the enormous amount of mucous emission goes about as a guard instrument against many pollutants which hold fast to the gills.

Extraordinary consideration and broad investigations have been assessing the enhancing properties of various algae against the heavy metal harming and radiation [6, 58]. Sayed *et al*. [8] underscored on the job of *Spirulina* food supplementation in enhancing the genotoxic harms actuated by arsenic in *O. niloticus*. Comparative discoveries were recorded by James *et al*. [59] utilizing various doses of *Spirulina* to neutralize harmful impacts of copper-induced *Labeo rohita.* Little is known with respect to the job of *Amphora* extract in improving the dangerous harms prompted by heavy metals in fishes disregarding its bioactive compounds (antioxidants, antiviral, antibacterial, and antifungal agents) recorded by GC-MS [23, 28, 60].

In the present investigation, *Amphora* bioactive activities improved the vast majority of the histopathological alterations in the kidney and gills of arsenic-exposed fish reflecting, thus, cytotoxic impacts. So, one can consider

that amphora can suppress free radicals liberated due to arsenic toxicity in the kidney and gills and thus preventing their deterioration. Such role of amphora is confirmed by El-Sayed *et al*. [23] under toxic impacts of paracetamol in liver tissue of rodents. In addition, the current centralization of carbohydrates and proteins may add to upgrade the antioxidative actions of *Amphora* species as hypothesized by Rupérez *et al*. [61] concentrating on different diatoms.

5-CONCLUSIONS

In one word, diatoms as *Amphora coffeaeformis* have organically active ingredients functioning as antibacterial, antioxidant, anti-fungal, antiviral, and anti-inflammatory adjacent to the superb amounts of proteins and carbohydrates which improve these substances. So it very well may be utilized as detoxification factor to alleviate the histopathological and histochemical impacts of the arsenic induced fishes.

Acknowledgements

I would like to take this opportunity to express sincere appreciation to Dr. Alaa El-Din H. Sayed, Assistant professor of Fish Biology, Zoology Department, Faculty of Science, Assuit University for supervision, continuous encouragement, reading manuscript and endless efforts during finishing.

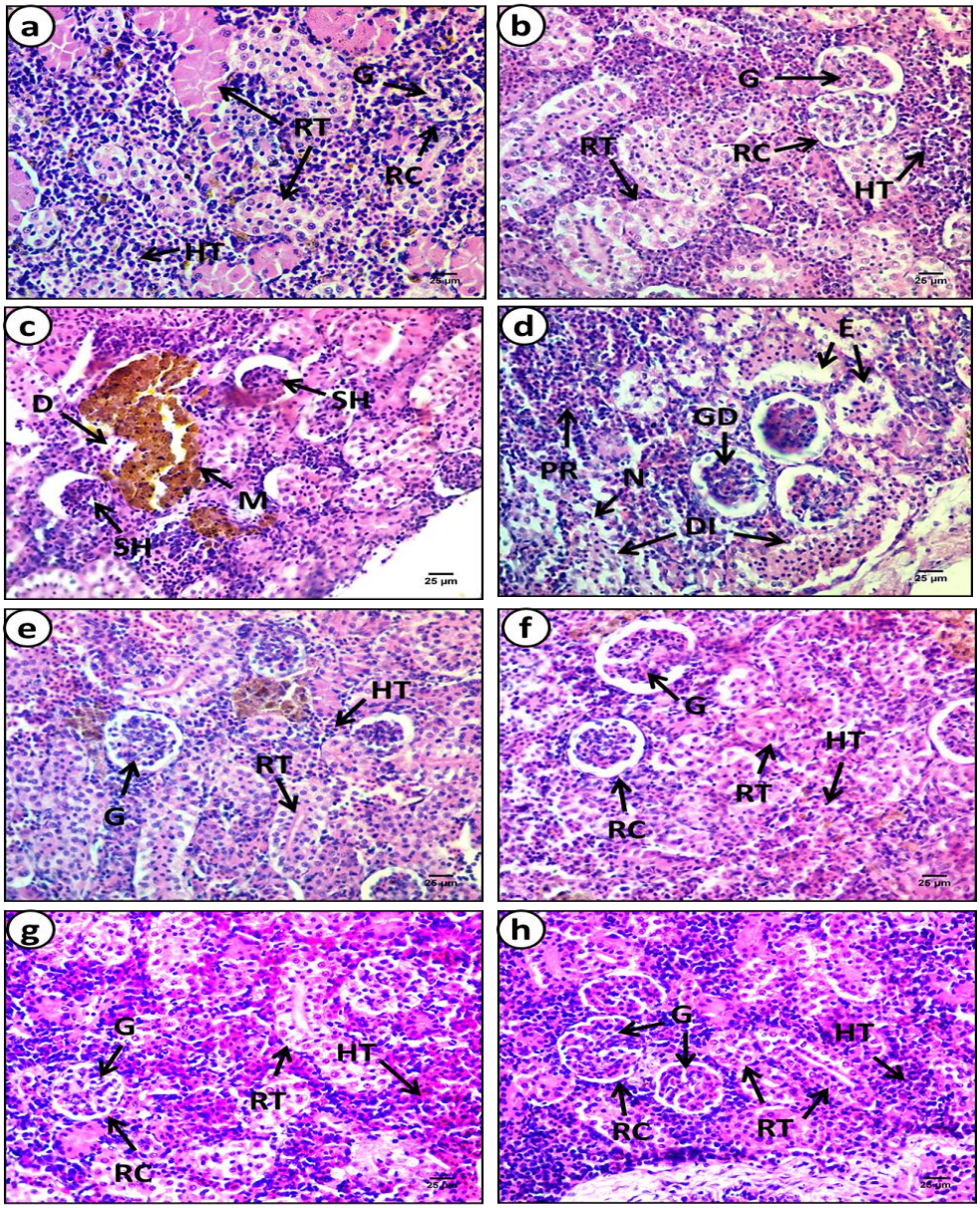

Fig. 1: Kidney sections of *Clarias gariepinus*: **(H&E, X 400)**: (a) Control and (b) Fish supplemented with amphora showing normal haemopoietic tissue (HT), glomerulus (G), renal corpuscle (RC) and renal tubule (RT). (c,d) Exposed to 19.2 mg/l and 38.3 mg/l arsenic showing melanomacrophages (M), necrosis (N), edema (E), shrinkage (SH) of glomerulus, glomerular deformation (GD), dissociation (DI) in the renal tubules and degeneration (D) plus proliferation (PR) in the haemopoitic tissue. (e) Exposed to 19.2 mg/l arsenic plus 7% amphora, (f) Exposed to 19.2 mg/l arsenic plus 10% amphora, (g) Exposed to 38.3 mg/l arsenic plus 7% amphora and (h) Exposed to 38.3 mg/l arsenic plus 10% amphora showing different shapes of improvement.

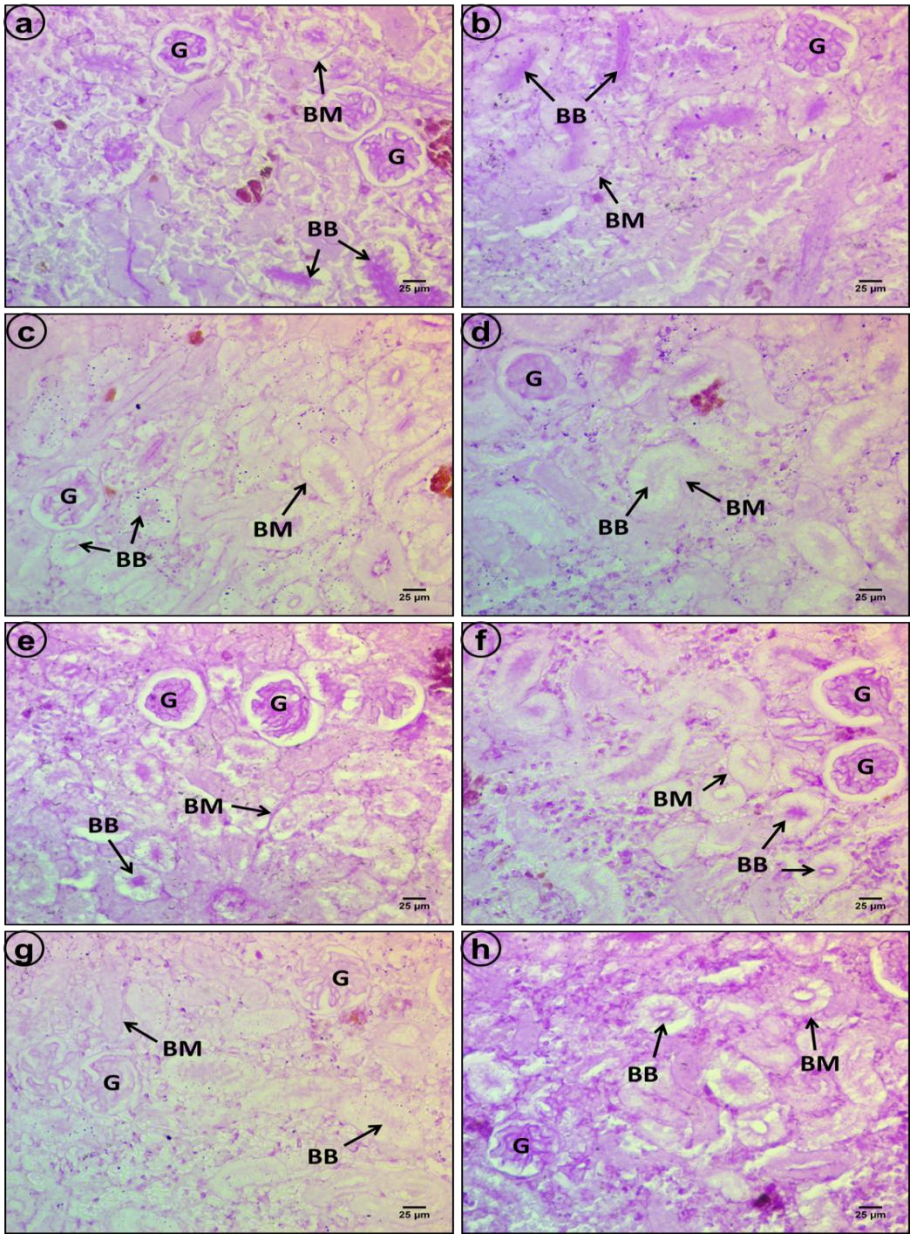

Fig. 2: Kidney sections of *Clarias gariepinus* **(PAS stain, X 400):** (a) Control and (b) Fish supplemented with amphora displaying marked PAS reactivity in the brush border (BB) and the basement membrane (BM) of the renal tubules and glomerulus (G). (c,d) Exposed to 19.2 mg/l and 38.3 mg/l arsenic showing a faint PAS reaction of carbohydrate materials in the different components of the kidney tissue. (e) Exposed to 19.2 mg/l arsenic plus 7% amphora, (f) Exposed to 19.2 mg/l arsenic plus 10% amphora, (g) Exposed to 38.3 mg/l arsenic plus 7% amphora and (h) Exposed to 38.3 mg/l arsenic plus 10% amphora showing different shapes of preservation of carbohydrate materials in the different components of the renal tissues.

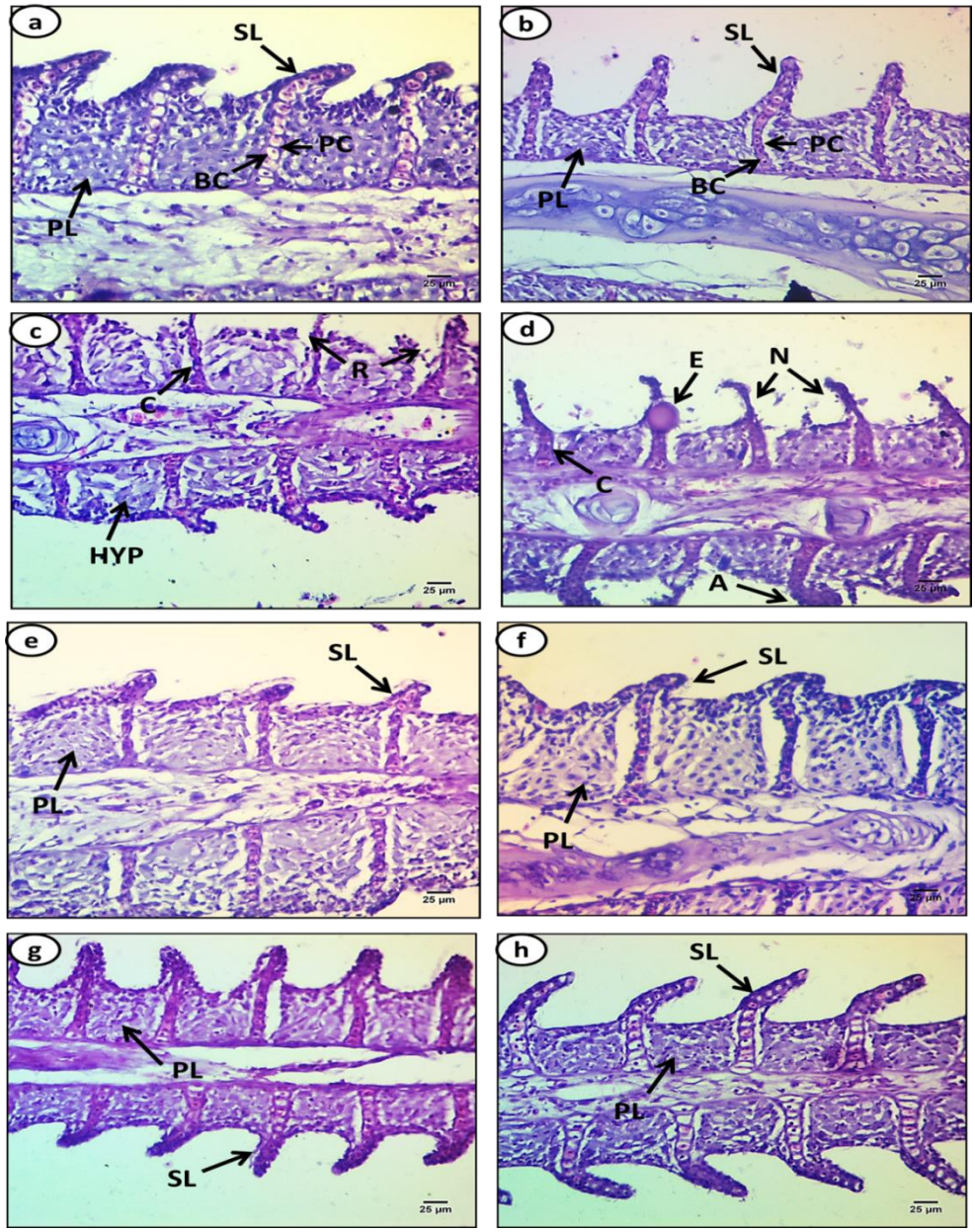

Fig. 3: Gills sections of *Clarias gariepinus*: **(H&E, X 400)**: (a) Control and (b) Fish supplemented with amphora showing the normal arrangement of the pillar cell - blood capillaries (PC-BC) system, normal primary (PL) and secondary lamellae (SL). (c,d) Exposed to 19.2 mg/l and 38.3 mg/l arsenic showing collapse (C) of the ladder-like arrangement of the (PC-BC) system, hyperplasia (HYP) of the epithelial cells, edema (E), aneurysm (A), necrosis (N) and rupture (R) in the lamellae. (e) Exposed to 19.2 mg/l arsenic plus 7% amphora, (f) Exposed to 19.2 mg/l arsenic plus 10% amphora, (g) Exposed to 38.3 mg/l arsenic plus 7% amphora and (h) Exposed to 38.3 mg/l arsenic plus 10% amphora showing different shapes of improvement.

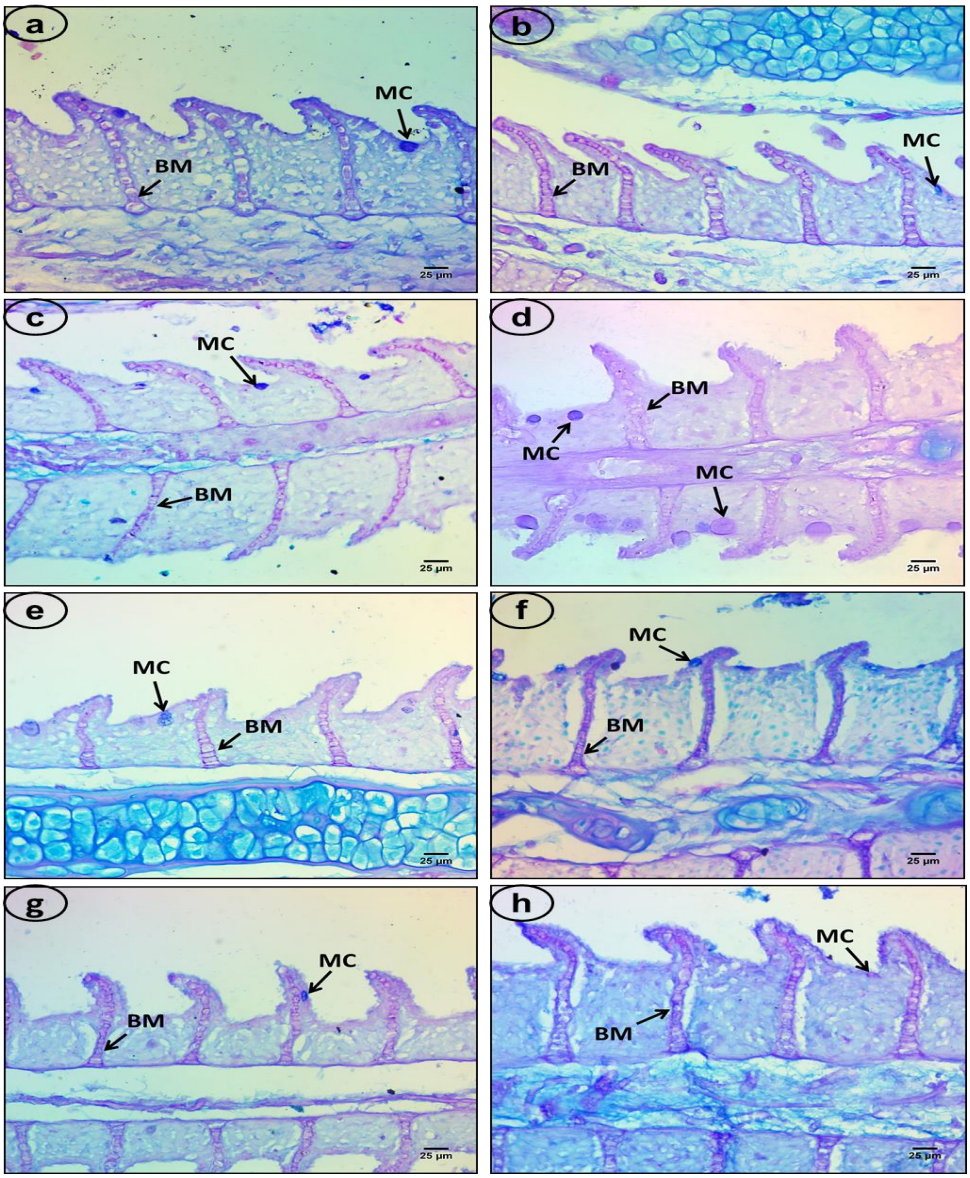

Fig. 4: Gills sections of *Clarias gariepinus* **(AB&PAS stain, X 400):** (a) Control and (b) Fish supplemented with amphora displaying a great coloration of the basement membrane (BM) covering the (PC-BC) system with normal number and size of mucous cells (MC) (c,d) Exposed to 19.2 mg/l and 38.3 mg/l arsenic showing a remarkable depletion of carbohydrate materials in the basement membrane (BM) with observed increasing in the number and size of mucous cells (MC). (e) Exposed to 19.2 mg/l arsenic plus 7% amphora, (f) Exposed to 19.2 mg/l arsenic plus 10% amphora, (g) Exposed to 38.3 mg/l arsenic plus 7% amphora and (h) Exposed to 38.3 mg/l arsenic plus 10% amphora showing different shapes of preservation of carbohydrate materials in the different components of the gill tissue with normal size and distribution of mucous cells (MC).

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الأمفورا تعدل الإضطرابات الهيستوباثولوجية والهستوكيميائية في الكلى والخياشيم لسمكة القرموط الأفريقي "كلارياس جاريبينس" المعرضة لسمية الزرنيخ

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من المعروف أن وصول الملوثات مثل المعادن الثقيلة إلى الوسط المائي يؤثر على مثل هذا المجال وعلى أشكال الحياة الحية مع المساعي لاستخدام العوامل المضادة للأكسدة لموازنة آثارها. كشف البحث الحالي عن الآثار الدفاعية المحتملة لمستخلص الأمفورا ضد أمراض الأنسجة والتعديلات الكيميائية النسيجية التي يسببها الزرنيخ في سمك القرموط الأفريقي. تعرضت الأسماك لجرعات تحت مميتة من الزرنيخ (١٩.٢ و ٣٨.٣ ملغم / لتر) لمدة ١٥ يوم. أظهرت أنسجة كلية الأسماك المعرضة للزرنيخ استسقاء الأنيبيبات وموت تحللى وانحلال النسيج المكون للدم وتجمعات الخلايا الصبغية الدفاعية واستسقاء محفظة بومان وانكماش الكبيبة ، بينما كشفت أنسجة خياشيم الأسماك المعرضة للزرنيخ عن تمزق صفائحي وتمدد الأوعية الدموية الصفائحية و تضخم واستسقاء الخلايا الطلائية وزيادة فى عدد الخلايا المخاطية. كشفت استجابة صبغة PAS الاستهلاك في المواد الكربوهيدراتية في الكلى والخياشيم في مجموعة الزرنيخ في المقابل مع الكنترول. تم تحسين هذه التغييرات التي يسببها الزرنيخ مع إضافة الأمفورا الغذائية بسبب مكوناتها النشطة طبيعياً والتي يمكن استخدامها كمضاد للأكسدة ومضاد للفيروسات ومضاد للبكتيريا ومضاد للالتهابات وكعامل لإزالة السموم.