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A protective effect of calcium carbonate against arsenic toxicity on the Nile catfish, *larias gariepinus*

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ABSTRACT

The objective of the present study was to test the protective upshot of calcium carbonate against the gifted toxicity of arsenic to the Nile cat-fish (*Clarias gariepinus*). Enhanced hepatosomatic index (HSI) and reduced gonadosomatic index (GSI) and intestinal index (ISI) as well as some of the tested blood parameters were recorded for fishes exposed to arsenic spotlight (1/10 & 1/20 LC_{50}). The plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, direct bilirubin, total lipids, glucose and total protein were significantly increased in fishes exposed to arsenic toxicity. Likewise, the activities of AST, ALT and lactate dehydrogenase (LDH) in the liver and muscles were radically increased, whereas the total protein and glycogen contents in these organs were significantly abridged following arsenic exposure; this may be an indication of energy expenditure attributable to arsenic toxicity. The histological examinations of the liver and gills renewed arsenic-induced degenerative changes of these organs. Furthermore, the addition of calcium carbonate as a limiting agent induces reversibility of most of these arsenic – induced changes, especially those of fishes subjected to 1/20 LC_{50} of arsenic. Consequently, calcium carbonate could be feasible to be used for the fortification of *C. gariepinus* in opposition to arsenic toxicity.

Keywords: Arsenic toxicity, *Clarias gariepinus*, calcium carbonate

INTRODUCTION

Levels of arsenic are higher in the aquatic environment than in most areas of land as it is fairly water-soluble and may be washed out of arsenic-bearing rocks (Edmonds and Francesconi, 1993). Recently, the anthropogenic activities such as treatment of agricultural land with arsenical pesticides, treating of wood using chromated copper arsenate, burning of coal in thermal plants power stations, and the operations of gold-mining have increased the environmental pervasiveness of arsenic and its rate of discharge into freshwater habitat (Pacyna *et al.*, 1995). Furthermore, arsenic is used broadly as sodium arsenite to control submerged aquatic vegetation in freshwater ponds and lakes (Roy and Bhattacharya, 2006). According to NAS (1977) 1.5-3.8 mg arsenite/L is effective and considered safe for fish. Many species of fish that live in arsenic polluted water contain arsenic between 1 - 10 g/g. At the bottom arsenic levels

in fish reported to be higher than 100 µg/g (Oladimeji *et al.*, 1984). The arsenic exists in the aquatic environment either in arsenite (As^{3+}) or arsenate (As^{5+}) form, which are interconverted through redox and methyaltion reactions (Bears *et al.*, 2006). The arsenate form is less toxic than arsenite one under *in vivo* and *in vitro* conditions (Cervantes *et al.*, 1994). Moreover, inside the cell these two forms react differentially with arsenite binding to sulphhydryl groups in the proteins and the arsenate disturb the process of phosphorylation (Andrew *et al.*, 2003). The arsenic toxicity may be related to the excess production of reactive oxygen species (ROS), namely superoxide ($O_2^{\circ-}$), hydroxyl ($OH^{\cdot}$), and peroxyl ($ROO^{\cdot}$) radicals and hydrogen peroxide (Hughes, 2002; Kitchin and Ahmed, 2003).

Fish are usually considered as organism of choice for assessing the effects of environmental pollution on aquatic ecosystem (Gernhöfer *et al.*, 2001). As the early identification of arsenic toxicity could be used as a hazard assessment tool, for this reason Bhattacharya and Bhattacharya (2007) developed a biomarker for arsenic exposure of Indian catfish (*Clarias batrachus*). The body indices as well as blood parameters of the fish have been used as indicators of environmental risk (Van der Oost *et al.*, 2003; Yang and Baumann, 2006; Datta *et al.*, 2007). Also, the assay of the enzymes activities (AST, ALT& LDH) in the blood and tissues of the fish frequently used as a diagnostic tool in human and animals (Bears *et al.*, 2006; Abdel-Hameid, 2007). Arsenic can also interfere with the fish immune system by suppressing antibody production (Ghosh *et al.*, 2007) as well as on lowering macrophage activity and maturation (Ghosh *et al.*, 2006). Several studies reporting arsenic- induced liver fibrosis, hepatocellular damage, inflammation, focal necrosis in addition to hepatocellular carcinoma (Liu *et al.*, 2001; ATSDR, 2002; Datta *et al.*, 2007).

Bears *et al.* (2006) indicate that fish can serve as vital indicators of arsenic toxicity as they are continuously exposed to arsenic through gill respiration and ingestion of arsenic- contaminated food. Although, the toxicity studies and the determination of the lethal concentration for 50% (LC_{50}) of fishes have been worked out in different fish species (Roy *et al.*, 2006; Ghosh *et al.*, 2006) but the effects of this pollutant on definite fish function systems are yet to be clarified (Datta *et al.*, 2007). In Egypt the Nile cat-fish (*C. gariepinus*), represents the second important fish species. Furthermore, in some countries they are the principal one. For these reasons, the present study was conducted to test the toxic effects of arsenic on body indices, blood parameters, carbohydrate metabolism and protein metabolism of the Nile catfish (*C. gariepinus*). Moreover, the study was extended to test the effect of arsenic on the histology of some vital organs (Liver and gills). It has been reported that the exposure of fishes to calcium relief the copper toxicity (de Vera and Pocsidio, 1998; Abdel-Tawwab *et al.*, 2007). So, the present undertaken verifies the protective effect of calcium carbonate against arsenic induced toxicity of *C. gariepinus.*

MATERIALS AND METHODS

1.Chemicals and preparations of stock solutions

Arsenous chloride was purchased from International office for trade service, Cairo, Egypt. Calcium carbonate, NaOH, and HCl were procured from El-Nasr pharmaceuticals chemical company, Abou-Zabel, Egypt. Also, clove oil was procured from Fura laboratory for cosmetics, Cairo, Egypt. Stock solution (100mM) of arsenous chloride was prepared following the method recommended by Datta *et al.* (2007). Stock solution 10% of $CaCO_3$ was used to maintain the desired concentration.

2.Fish

Adult male *C. gariepinus* ranging 23.1 ± 3.0 cm in total length and weighing 48.1 ± 5.2 g were gifted from Central Laboratory of Aquaculture, Abbassa, Abou-Hammad, Sharkia, Egypt. The fish used in this study were apparently healthy. Fish were acclimatized at laboratory conditions for one week in indoor tanks in well aerated tap water (Water temperature 25-27˚C; PH 7.2-7.5). Fish were fed on adlibitum (3% of total body weight) with minced chicken liver with a commercially available fish feed. The water of the aquaria was renewed every 24 h to eliminate the faecal parts as well as the soluble excretory products. Fish handling was done carefully following the standard laboratory practice.

3.Determination of LC_{50} for arsenic

The 96 h median tolerance limit (96 h LC_{50}) was determined (at a static condition) by exposing the fish to five ascending concentrations of arsenous chloride. Cumulative mortality was determined after 96 h; the dead fish was removed once it is observed. The 96 h LC_{50} (89mg/l) was determined by graphically plotting the percentage mortality versus the arsenous chloride concentrations (Fig. 1).

1.Experimental groups

The experiment was conducted at a static system in glass aquaria measuring 75 cm length, 29 cm width and 40 cm height. The fish were distributed into 9 experimental groups; each consisting of 5 fish. The experimental groups were categorized as follows:-

Group 1: Fish reared in dechlorinated tap water (control).
Group 2: Fish exposed to 1/10 LC_{50} of arsenic.
Group 3: Fish exposed to 1/20 LC_{50} of arsenic.
Group 4: Fish exposed to further 50 mg/l CaCO3 and zero arsenic.
Group 5: Fish exposed to further 50 mg/l CaCO3 and 1/10 LC_{50} of arsenic.
Group 6: Fish exposed to further 50 mg/l CaCO3 and 1/20 LC_{50} of arsenic.
Group 7: Fish exposed to further 100 mg/l CaCO3 and zero arsenic.
Group 8: Fish exposed to further 100 mg/l CaCO3 and 1/10 LC_{50} of arsenic.
Group 9: Fish exposed to further 100 mg/l CaCO3 and 1/20 LC_{50} of arsenic.

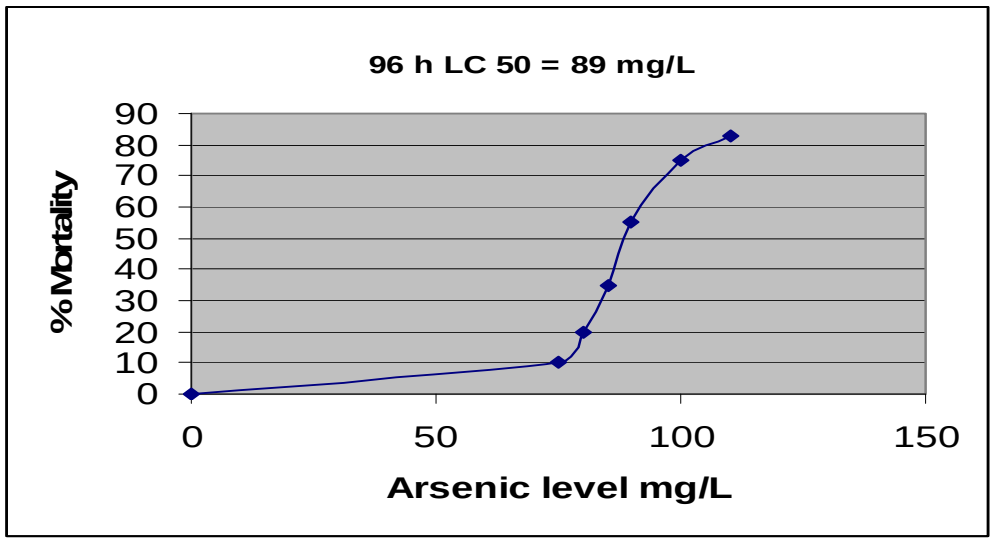

Fig. (1): Graphical estimation of 96 h LC_{50} of arsenous chloride to *C. gariepinus*.

4. Somatic indices

For each experimental group, the liver, gonad, intestine and the fish without the gut (gutted weight) were weighed. The hepatosomatic index (HSI), gonadosomatic index (GSI) and intestinosomatic index (ISI) was computed as a ratio of the organ weight to the gutted weight.

5. Haematological parameters

After 20 days from the beginning of the experiment, the fish were collected and anesthetized following the method of Ribas *et al.* (2007) using 1ml/l clove oil. The blood samples were collected with hypodermic syringe from the caudal vessels. Then, it was transferred to lithium heparinized tube to prevent blood clotting. By using Neubauer haemocytometer slide, the RBCs were counted after diluting the blood with saline solution (0.75% NaCl). The Hct values were determined by sucking the blood into microhaematocrit capillary tubes and then it were centrifuged for 5 min in a microhaemotocrit centrifuge. The Hct were computed as a ratio of packed cell volume to total blood volume. The blood haemoglobin content (Hb, g/100ml) was determined following the method recommended by Henry (1964). The remained blood were centrifuged at 5000 rpm for 5 min, the blood plasma were carefully separated into ependorf tube and stored in deep freezer (-20°C) till analysis.

6. Biochemical analysis

From each fish, the liver and the white epiaxial muscles were secluded. The tissues were homogenized in cold distilled water using glass homogenizer. The tissue homogenates were centrifuged twice at 4000 rpm for 5 min. The tissue supernatants were separated to be used for the determination of enzymes activities and metabolites contents. The AST (EC 2.6.1.1) and ALT (EC 2.6.1.2)

activities were assayed colorimetrically following the method of Reitman and Frankel (1957), in plasma and tissue. The activity of LDH (EC 1.1.1.27) was kinetically assayed at 340nm (Hochachka *et al.*, 1978) in tissue only. Total and direct bilirubin (Malloy and Evelyn, 1937), total lipids (Schmit, 1964), glucose (Trinder, 1969) and total proteins (Henry, 1964) were determined in blood plasma. The glycogen content in muscles and liver were determined by alkaline digestion of the tissue (Oser, 1979) followed by enzymatic measurement of glucose.

7. Histology

The liver and the gills of the dissected fish were excised out of the fish. Small pieces of these organs were fixed in neutral buffered formalin, dehydrated and embedded in paraffin. Tissues sections (6 μm) were stained with haematoxylin and eosin. Photographs of the stained tissue sections were captured using trinocular light microscope (Bio-Med) and attached with soft ware. Image-pro plus for windows (8484 Georgia Avenue, Silver Spring, Maryland, USA). Degree of tissue change (DTC) was used to evaluate semi-quantitatively the severity of tissue lesions. The alterations in the studied organs were classified in progressive orders as follows: Stage I, which do not change the normal functioning of the tissue; stage II, which are more severe and disrupt the normal function of the tissue; and stage III, which are very severe and induce irreparable tissue damage. By screening the number of tissue lesions in stages I, II, and III, for each animal, the DTC value was calculated by the formula: DTC = $(1\times\Sigma\ I) + (10\ \Sigma\ II) + (100\times\Sigma\ III)$. The values of DTC ranged from 0-10 indicate no damage of the organ; 11-20 indicate slight damage to the organ; 21-50 indicate moderate changes in the organ; 50-100 indicate severe damage and more than 100 indicate irreversible damage to the organ (Poleksic and Mitrovic-Tutundzic, 1994; Simonato *et al.*, 2008).

8- Statistical analysis

The data of this work were presented as means ±standard deviations. Statistical analysis of the effect of arsenic and CaCO3 was done using two-way ANOVA followed by Duncan's multiple range test as a post-hoc test to compare between means at $P\leq0.05$ (Pipkin, 1984). Tests were carried out using software SPSS 11.

RESULTS

1. Somatic indices

The values of HSI were significantly increased, whereas those of GSI and ISI were significantly reduced in the fishes exposed to both arsenic levels (8.9 &4.5 mg/l). It was found that these changes were dose dependent. Exposure of fishes to further calcium carbonate (50 or 100 mg/l) did not induce any significant changes of the tested somatic indices. Compared to the control fish, the somatic indices did not differ significantly in fishes subjected to both tested levels of calcium carbonate along with examined arsenic levels (Table 1).

Table (1). Changes in hepatosomatic index (HSI, %), gonadosomatic index (GSI, %) and intestinosomatic index (ISI, %) of *C. gariepinus* exposed to two sublethal concentrations of arsenic (As) at two water hardness levels for 20 days.

Groups	HSI	GSI	ISI
Control	0.551±0.023 a	0.312±0.049 a	7.291±0.423 a
1/10 LC_{50} of As	0.836±0.031 b	0.174±0.017 b	6.124±0.621 b
1/20 LC_{50} of As	0.646±0.056 c	0.245±0.071 c	6.561±0.742 c
50 mg/l CaCO3	0.591±0.072 a	0.291±0.042 a	7.123±0.562 a
50 mg/l CaCO3 + 1/10 LC_{50} of As	0.660±0.042 c	0.284±0.042 a	6.924±0.452 a
50 mg/l CaCO3 + 1/20 LC_{50} of As	0.585±0.067 a	0.324±0.092 a	7.492±0.821 a
100 mg/l CaCO3	0.574±0.027 a	0.320±0.121 a	7.801±0.721 a
100 mg/l CaCO3 + 1/10 LC_{50} of As	0.571±0.022 a	0.304±0.049 a	7.321±0.561 a
100 mg/l CaCO3 + 1/20 LC_{50} of As	0.585±0.054 a	0.294±0.072 a	7.421±0.762 a

All data expressed as mean of 5 fishes ±standard deviation.
Means in the same column that do not share letters are significantly different at P≤0.05.

2. Blood parameters

Compared to fish of the control group, the RBCs counts, Hb contents and Hct values were significantly reduced due to exposure to both the tested arsenic levels (Table 2). The tested blood parameters of the fish, either exposed to calcium carbonate individually or in mixture of arsenic at two levels did not exhibit significant differences from those of the control group.

Table (2). Changes in some blood parameters *C. gariepinus* exposed to two sublethal concentrations of arsenic (As) at two water hardness levels for 20 days.

Groups	RBCs ($X10^6$/mm3)	Hb (g/l)	Hct (%)
Control	2.124±0.121 a	90.421±5.120 a	25.513±1.123 a
1/10 LC_{50} of As	1.421±0.215 b	71.412±8.125 b	18.149±1.052 b
1/20 LC_{50} of As	1.6214±0.425 b	80.120±6.240 b	20.389±2.256 b
50 mg/l CaCO3	2.121±0.231 a	91.023±6.121 a	25.670±2.490 a
50 mg/l CaCO3 + 1/10 LC_{50} of As	1.954±0.316 a	85.431±6.132 a	23.124±1.624 ab
50 mg/l CaCO3 + 1/20 LC_{50} of As	2.121±0.215 a	89.124±7.181 a	24.769±1.351 a

. All data expressed as mean of 5 fishes ±standard deviation.
. Means in the same column that do not share letters are significantly different at P≤0.05.

3. Plasma biochemical parameters

All the tested plasma biochemical parameters of fish exposed to arsenic, which include enzymes activities (AST & ALT) and metabolites level (Total bilirubin, total lipids, glucose & total proteins), were increased significantly over those recorded for the control group (Table 3). The effect was correlated with the arsenic level. The parameters of the fish exposed to the tested levels of calcium carbonate alone and to those exposed to mixture of other 50 mg/l calcium carbonate and 1/20 LC_{50} of arsenic were not significantly differed from those of the control. In contrast to the aforementioned result, all of the examined biochemical parameters for fish exposed to additional 50 or 100 mg/l calcium carbonate along with 1/10 LC_{50} of arsenic were significantly increased over those of the control. The exposure to the mixture of supplementary 100 mg/l calcium carbonate and 1/20 LC_{50} of arsenic resulted in non significant differences of the tested biochemical parameters, except for the plasma glucose level which was significantly increased.

4. Carbohydrate metabolism

The LDH activity in the liver of fish exposed to arsenic was significantly higher than those recorded for the control group (Table 4). Similarly, the LDH activity in the muscles was increased due to arsenic exposure. The increase in its activity was significantly higher in fish exposed to 1/10 LC_{50} of arsenic, whereas it was non- significantly different in case of fish exposed to 1/20 LC_{50} of arsenic. The LDH activities in the liver and muscles were non-significantly changed in the fish subjected to both spare calcium carbonate levels. The use of calcium carbonate both tested levels exposure along with 1/20 LC_{50} of arsenic induces non- significant difference of LDH activities in the examined tissues. Controversially, the LDH activities in the liver and muscles of fish exposed to the mixture of calcium carbonate (Both tested levels) and 1/10 LC_{50} of arsenic were significantly higher than those of the control group. Both examined arsenic levels induced significantly lower liver and muscle glycogen contents. The use of additional calcium carbonate 50mg/l along with lower arsenic level induced non-significant changes of the liver and muscle glycogen contents. On the other hand, its use in conjunction with higher level of arsenic resulted in significantly lower glycogen contents in the prescribed tissues. The use of the highest level of calcium carbonate in conjunction with both arsenic levels induced reversibility of the liver and muscle glycogen contents. Only, the muscle glycogen of fish exposed to higher levels of both calcium carbonate and arsenic was significantly reduced.

Table (3). Changes in some biochemical parameters in blood plasma of *C. gariepinus* exposed to two sublethal concentrations of arsenic (As) at two water hardness levels for 20 days.

Groups	AST (U/l)	ALT (U/l)	Total bilirubin (mg/l)	Direct bilbirubin (mg/l)	Total lipids (g/l)	Glucose (g/l)	Total proteins (g/l)
Control	81.243 ±2.121 a	54.342 ±1.256 a	1.861 ±0.126 a	1.021 ±0.245 a	17.340 ±0.920 a	0.879 ±0.033 a	18.426 ±1.403 a
1/10 LC_{50} of As	120.123 ±4.651 b	85.627 ±6.235 b	2.912 ±0.123 b	1.852 ±0.563 b	23.460 ±1.260 b	1.124 ±0.052 b	22.469 ±1.067 b
1/20 LC_{50} of As	97.435 ±7.623 c	65.462 ±3.921 c	2.301 ±0.073 c	1.541 ±0.423 b	21.560 ±0.832 c	1.034 ±0.046 c	20.451 ±1.123 b
50 mg/l CaCO3	84.621 ±8.235 a	56.231 ±5.162 a	1.723 ±0.213 a	1.123 ±0.423 a	17.630 ±0.693 a	0.901 ±0.066 ad	18.721 ±0.947 a
50 mg/l CaCO3 + 1/10 LC_{50} of As	99.241 ±6.236 c	71.212 ±4.592 c	2.362 ±0.201 c	1.561 ±0.521 b	20.192 ±0.892 c	0.981 ±0.069 d	19.02 ±2.102 a
50 mg/l CaCO3 + 1/20 LC_{50} of As	84.271 ±3.921 a	56.421 ±4.583 a	1.908 ±0.143 a	1.374 ±0.673 ab	18.041 ±0.672 a	0.921 ±0.092 ad	18.981 ±0.947 a
100 mg/l CaCO3	83.462 ±5.923 a	55.923 ±6.121 a	1.902 ±0.261 a	1.213 ±0.369 a	17.943 ±1.291 a	0.911 ±0.072 a	18.942 ±1.041 a
100 mg/l CaCO3 + 1/10 LC_{50} of As	103.692 ±6.426 c	75.121 ±9.216 c	2.421 ±0.321 c	1.621 ±0.324 b	21.453 ±1.326 bc	1.031 ±0.074 c	21.862 ±1.469 b
100 mg/l CaCO3 + 1/20 LC_{50} of As	83.627 ±5.024 a	56.172 ±7.921 a	2.046 ±0.456 a	1.131 ±0.421 a	18.023 ±2.762 a	0.945 ±0.072 d	19.146 1.123 a

All data expressed as mean of 5 fishes ±standard deviation.
Means in the same column that do not share letters are significantly different at P≤0.05.

Table (4): Changes in the activity of LDH (U/min/g fresh tissue) and glycogen content (mg glucosyl glucose / g fresh tissue) in liver and muscle of *C. gariepinus* exposed to two sublethal concentrations of arsenic (As) at two water hardness levels for 20 days.

	LDH		Glycogen	
Group	Liver	Muscle	Liver	Muscle
Control	2.452 ±0.236 a	105.243 ±4.563 a	1.361 ±0.035 a	0.516 ±0.029 a
1/10 LC_{50} of As	3.185 ±0.314 b	145.412 ±5.129 b	0.953 ±0.029 b	0.458 ±0.046 b
1/20 LC_{50} of As	2.946 ±0.261 b	109.374 ±6.274 a	1.167 ±0.041 c	0.495 ±0.032 ab
50 mg/l CaCO3	2.414 ±0.394 a	107.139 ±7.149 a	1.349 ±0.046 a	0.498 ±0.089 ab
50 mg/l CaCO3 + 1/10 LC_{50} of As	2.853 ±0.426 b	131.721 ±6.293 c	1.224 ±0.051 c	0.408 ±0.056 b
50 mg/l CaCO3 + 1/20 LC_{50} of As	2.568 ±0.367 a	110.426 ±7.014 a	1.314 ±0.063 a	0.499 ±0.067 ab
100 mg/l CaCO3	2.638 ±0.528 ab	106.943 ±6.981 a	1.328 ±0.072 a	0.521 ±0.078 a
100 mg/l CaCO3 + 1/10 LC_{50} of As	2.863 ±0.423 b	126.166 ±7.142 c	1.301 ±0.057 a	0.453 ±0.049 b
100 mg/l CaCO3 + 1/20 LC_{50} of As	2.416 ±0612 a	109.624 ±7.753 a	1.402 ±0.082 a	0.497 ±0.066 ab

All data expressed as mean of 5 fishes ±standard deviation.
Means in the same column that do not share letters are significantly different at P≤0.05.

5. Protein metabolism

The AST and ALT activities were significantly privileged in fishes exposed to both tested arsenic levels (Table 5). Fish exposed to both tested levels of calcium carbonate have AST and ALT activities, which were not significantly deviated from those recorded for control fish. On the other hand, the AST and ALT activities in liver and muscle of the fish exposed to the mixture of tested calcium carbonate levels and 1/20 LC_{50} of arsenic were retained to the normal activity levels. Controversially, the AST in liver and muscles and muscle ALT recorded appreciably superior activities in fish exposed to mixture of added calcium carbonate levels and 1/10 LC_{50} of arsenic. The ALT activity in the liver of fish exposed to 100 mg/l calcium carbonate in conjunction with 1/10 LC_{50} of arsenic was non-significantly differed from those recorded for the control group.

The exposure to arsenic induces significant reduction of the total proteins content in the examined fish tissues, whereas the exposure to both tested levels of calcium carbonate induces non-significant effect. The exposure to both levels of calcium carbonate along with both levels of arsenic induces weak reversibility of the total proteins content in the liver. Only, those of fishes exposed to the lowest level of calcium carbonate and the highest arsenic level was significantly lower than those of the control.

Table (5). Changes in AST and ALT activities (U/min/g fresh tissue) and total proteins content (mg/g fresh tissue) in the liver and muscle of *C. gariepinus* exposed to two sublethal concentrations of arsenic (As) at two water hardness levels for 20 days.

Groups	AST		ALT		Total proteins	
	Liver	Muscle	Liver	Muscle	Liver	Muscle
Control	48.432 ±5.121 a	25.625 ±1.432 a	40.592 ±6.927 a	20.463 ±0.982 a	90.465 ±8.241 a	21.928 ±0.592 a
1/10 LC_{50} of As	65.513 ±4.152 b	33.156 ±1.914 b	62.692 ±4.212 b	29.614 ±1.842 b	60.246 ±9.356 b	15.331 ±1.014 b
1/20 LC_{50} of As	58.724 ±5.136 b	29.723 ±1.634 c	51.937 ±5.214 c	25.147 ±2.361 c	70.861 ±6.725 c	17.853 ±1.126 c
50 mg/l CaCO3	50.312 ±6.724 a	26.481 ±1.378 a	42.371 ±4.125 a	21.793 ±2.146 a	92.645 ±9.325 a	20.784 ±1.214 a
50 mg/l CaCO3 + 1/10 LC_{50} of As	60.261 ±5.243 b	30.482 ±2.163 bc	55.792 ±4.132 c	26.615 ±1.792 bc	70.149 ±7.632 c	17.124 ±1.146 c
50 mg/l CaCO3 + 1/20 LC_{50} of As	51.429 ±6.241 a	26.372 ±2.017 a	45.629 ±6.731 a	22.069 ±1.998 a	84.937 ±7.642 a	20.982 ±1.219 a
100 mg/l CaCO3	52.371 ±5.432 a	25.894 ±2.146 a	44.671 ±5.291 a	21.137 ±1.984 a	92.561 ±9.146 a	20.947 ±1.461 a
100 mg/l CaCO3 + 1/10 LC_{50} of As	61.425 ±4.938 b	29.824 ±2.263 c	45.463 ±5.241 a	25.431 ±2.413 bc	80.426 ±6.293 a	16.894 ±2.143 c
100 mg/l CaCO3 + 1/20 LC_{50} of As	52.604 ±7.125 a	27.093 ±2.087 a	43.921 ±4.231 a	22.101 ±2.141 a	85.739 ±7.426 a	20.024 ±1.635 a

All data expressed as mean of 5 fishes ±standard deviation.
Means in the same column that do not share letters are significantly different at $P \leq 0.05$.

6. Histological alterations

After the exposure to both arsenic levels, the liver cells were found to lose their regular shape due to partial precipitation of both cytoplasmic and nuclear material, resulting shape deformation, vacuolization, necrosis and even cell damage (Table 6 and Fig. 2). Also, the gill hyperplasia was found the most induced branchial changes due to arsenic exposure (Table 6 and Fig. 3).

The DTC values recorded for the liver of arsenic exposed fish were significantly higher than those recorded for the control group, with a mean values of 54.79 (severe damage) and 31.46 (moderate damage) for fishes exposed to 1/10 and 1/20 LC50 of arsenic, respectively (Table 7). Moderate damage for the gills of fish exposed to tested levels of arsenic was reported from the mean DTC values (40.26 and 24.72). The DTC values for the liver of fish exposed to both calcium carbonate levels did not significantly differed from those recorded for the unexposed fish. DTC value for the gill of fish exposed to additional 50 mg/l calcium carbonate lie within the normal range, whereas those recorded for fish exposed to extra 100 mg/l calcium carbonate was 14.86 (slight damage). The exposure to further 50 mg/l calcium carbonate along with 1/20 LC50 of arsenic resulted in non-significant differences of the liver and gills DTC values, when compared with respective control. The same is spot on for the liver of fish exposed to added 100 mg/l calcium carbonate a long with 1/20 LC50 of arsenic. Significantly higher DTC values for the gills were recorded with average 33.14 (moderate damage) and 17.56 (Slight damage) for fishes exposed to more 100 mg/l calcium carbonate alongside with 1/10 and 1/20 LC50 of arsenic, in that order.

Table (6). Histological alterations found in the liver and gills of *C. gariepinus* exposed to two sublethal concentrations of arsenic (As) at two water hardness levels for 20 days.

Liver	Gills
Stage I -Nuclear hypertrophy -Cellular hypertrophy -Cytoplasmic vacuolation -Nuclear atrophy -peripheral nuclei	-Hyperplasia of gill epithelium -Epithelial lifting of gill lamellae -Lamellar fusion
Stage II -Cytoplasmic degeneration -Cell rupture -Nuclear degeneration	-Rupture of epithelial cells with haemorrhage -Comlete fusion of lamellae -Rupture of pillar cells

Table (7) : Degree of tissue change (DTC) for liver and gills of *C. gariepinus* exposed to two sublethal concentrations of arsenic (As) at two water hardness levels for 20 days.

Group	Liver	Gills
Control	14.62±1.12 a	9.71±1.52 a
1/10 LC_{50} of As	54.792±5.34 b	40.26±5.19 b
1/20 LC_{50} of As	21.46±3.69 c	24.72±4.27 c
50 mg/l CaCO3	14.49±3.69 a	12.48±4.85 af
50 mg/l CaCO3 + 1/10 LC_{50} of As	24.13±5.17 c	25.14±6.13 c
50 mg/l CaCO3 + 1/20 LC_{50} of As	16.73±5.32 a	14.98±4.68 d
100 mg/l CaCO3	12.12±4.29 a	19.86±5.97 d
100 mg/l CaCO3 + 1/10 LC_{50} of As	20.76±5.82 c	33.14±3.96 e
100 mg/l CaCO3 + 1/20 LC_{50} of As	15.01±3.14 a	17.56±6.12 f

All data expressed as mean of 5 fishes ±standard deviation.
Means in the same column that do not share letters are significantly different at P≤0.05.

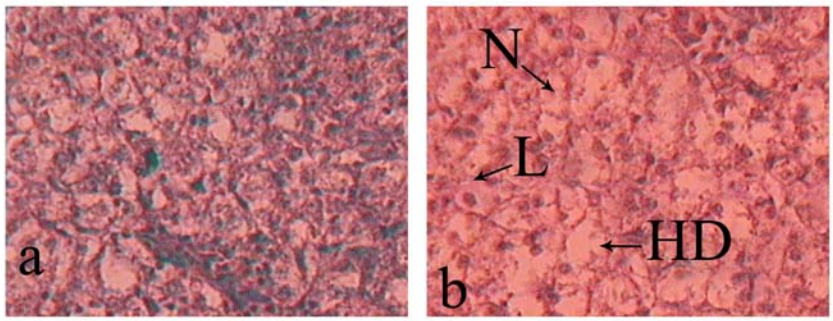

Fig. (2). Photomicrographs.Section in liver of control *C. gariepinus (a) and exposed to* 1/10 LC50 of As for 20 days (b) showing necrosis (N),enlarged hepatocyte (L) and hepatocyte degeneration (HD) (haematoxylin and eosin 400X).

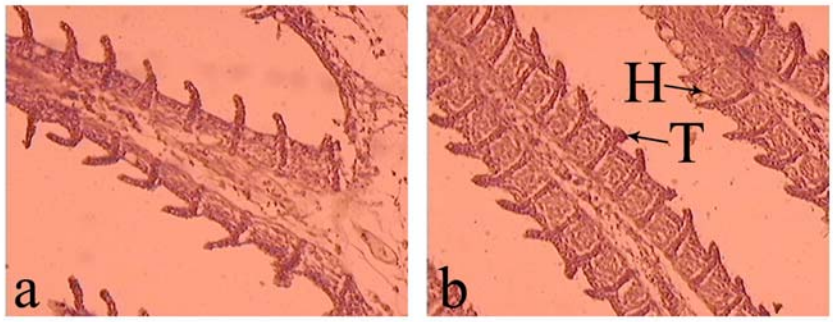

Fig. (3). Photomicrographs.Section in gills of control *C. gariepinus (a) (100x) and exposed to* 1/10 LC50 of As for 20 days (b) showing hyperplasia (H) of the gills epithelium and telangiectiasis (T) (haematoxylin and eosin 40X).

DISCUSSION

The 96-h LC_{50} of arsenous chloride recorded in the present study was found to be 89 mg/L for *C. gariepinus*, which is close to 48-h LC50, 84 mg/l for *C. batrachus* (Bhattacharya and Bhattacharya, 2007) and 76 mg/L for *Channa punctatus* (Roy and Bhattacharya, 2006). Therefore, *C. gariepinus* is a sensitive species and the assessment of the early effects of arsenic exposure on this fish may explore the mechanism of its toxicity. Then, Liu *et al.* (2008) recorded 56 mg/l as a 96-h LC50 of arsenate for Zebrafish (*Danio rerio*). This value is quite different from the 96-h LC50 reported in the present study; this may be related to species differences and genetic relationship. Thus, arsenic exposure is so dangerous for wide-scale of organisms including fish and human, and wildlife (ATSDR, 2002). Few research works were dealing with treatment of arsenic toxicity. Bhattacharya and Bhattacharya (2007) found that intravenous pretreatment with N-acetylcysteine delay mortality of *C. batrachus* by 10 days and induce reversibility of arsenic induced changes. The second work was done by Liu *et al.* (2008), they tested the interactive effect of other contaminant as perchlorate on arsenic toxicity. They found that Zebrafish (*Danio rerio*) pretreated with perchlorate have lower LC50 value (43 mg/l) than those subjected to arsenate only (56 mg/L). Thus, the perchlorate exposure enhances arsenate toxicity to Juvenile Zebrafish. This is consistent with the hypothesis that hypothyrodism induced by perchlorate enhances the toxicity of arsenate (Theodorakis *et al.*, 2006; Liu *et al.*, 2008) which was reported to synergetic to the antioxidant defense system (Konukoğu *et al.*, 1998). This may inspire arsenic toxicity as arsenic stimulates oxidative stress (Liu *et al.*, 2001). Then again, research experiments found that the fish exposures to calcium carbonate shield the fish from metal toxicity (Dutta and Kaviraj, 1996; de Vera and Pocsidio, 1998; Abdel-Tawwab and Mousa 2005; Abdel-Tawwab *et al.* 2007). Likewise, the elevated dietary calcium reduces water borne cadmium uptake of rainbow trout and hence reduces the susceptible toxicity of Cd (Baldisserotto *et al.*, 2004).

Although the body indices are quite general and non-specific, but their low cost and ease still make them valuable environmental risk assessment tool (Van der Oost *et al.*, 2003). In the present study the recorded enhancement of HSI values due to arsenic exposure indicates degenerative changes in the liver. This also may enlighten hepatomegaly and metal accumulation in the liver. Hepatomegaly were previously reported for fish subjected to various pollutants (Pait and Nelson, 2003; Barse *et al.*, 2006; Abdel-Hameid, 2007; Datta *et al.*, 2007).

In the present study, the arsenic reduced GSI values for *C. gariepinus*. This was formerly recorded by Yamaguchi *et al.* (2007) for male catfish *(Pangasianodon hypophthalamus*) subjected to metal contaminated water. They reported necrosis of spermatogonia and vacuolated Sertoli cells of the exposed fish.

The current experiment recorded reduction of ISI values after arsenic exposure. This result is in union with those reported for other pollutants (Abdel-Hameid, 2007; Abdel-Tawwab *et al.*, 2007). This may be due to loss of appetite and concomitantly resulted in reduction of total body weight. Several research works were used the changes in ISI as indicator of physiological conditions and body loss caused by reduced feed intake (Abdel-Tawwab *et al.*, 2006; Abdel – Hameide, 2007; Abdel-Tawwab *et al.*, 2007). The exposure of *C. gariepinus* in the present study to calcium along with arsenic causes a reversibility of the tested somatic indices. These results met those reported by Abdel-Tawwab and Mousa (2005) and Abdel-Tawwab *et al.* (2007).

The changes in the haematological parameters of fish are a helpful biomarker for evaluating their health status (Řehulka *et al.*, 2004). The arsenic induced reduction in the blood parameters recorded in the present study. This may be due to haemolysis and/or haemorrhage caused actions of pollutants to the fish (Alkindi *et al.*, 1996; Simonato *et al.* 2008).

The fish liver is one of the sensitive organs in which various metabolic pathways take place. Therefore, the effects of a chemical usually appear primarily in the liver (Roy and Bhattacharya, 2006). Liver function tests have been used as indicators to access alterations in liver functioning following exposure to arsenic (Roy and Bhattacharya, 2006). In the present study plasma AST, ALT, bilirubin, total lipids and total proteins were used as indicator of arsenic-induced hepatotoxicity. The recorded data revealed elevated levels of these parameters after arsenic exposure. This may reflect liver damage due to arsenic toxicity (Yang and Chen, 2003; Roy and Bhattacharya, 2006; Datta *et al.*, 2007). The observed rise in bilirubin level might be either due to haemolysis or due to turbulence in the uptake and conjugation of bilirubin by the hepatocytes (Datta *et al.*, 2007). Furthermore, the recorded marked rise of the plasma total lipids is in concurrence with those previously reported for other pollutants (Saeed, 1989; Arias, 1990; Diab *et al.*, 1996). This may imitate certain degree of the effect of toxic agents and environmental pollutants. The hyperglycemia recorded in the present study after arsenic exposure may be an indication of induced degenerative changes in the hepatopancreas. This result agree with those reported by Roy and Bhattacharya (2006) who recorded necrosis of hepatopancreas of *Channa punctatus* bare to arsenic. In the present study, the use of calcium carbonate induced reversibility of most of tested plasma biochemical parameters for fishes exposed to $1/_{20}$ LC_{50} of arsenic. In contrary, these items exhibited non-reversibility in case of the use of calcium carbonate along with $1/_{10}$ LC_{50} of arsenic. This could possibly explain as the arsenic toxicity depends not only on the dose but also on the species (Bhattacharya and Bhattacharya, 2007). Thus, the use of additional calcium carbonate could be constructive as a protective agent against the toxicity of low arsenic level (1/20 LC_{50} or low).

The revelation to arsenic causes reductions in liver and muscle glycogen content. This may reflect high energy utilization due to stress induced by pollutants (Dangè, 1986). It could also possibly due to that high energy demand requires for the synthesis of detoxifying enzymes (Begum and Vijayaraghavan, 1995; Hori *et al.*, 2006). The induction of LDH activity in fish exposed to arsenic observed in this study could possible reflects the high rate of conversion of lactate to pyruvate and then to glucose. This result was previously reported for other fish species exposed to phenol pollution (Hori *et al.*, 2006; Abdel-Hameid, 2007). Bhattacharya and Bahattacharya (2007) reported that arsenic exposure increases the production of H_2O_2 may lead to oxidative stress.

The amplified activities of AST and ALT in liver and muscle of *C. gariepinus* due to arsenic publicity indicating enhanced tissue proteolysis. For this reason the total protein content recorded reduced level. The tissue proteolysis was previously reported for different fish species subjected to pollution (Dangè, 1986; Abdel-Hameid, 1994; Barse *et al.*, 2006; Hori *et al.*, 2006). This could probably enlighten the use of protein as an alternative source of energy due to high energy demand that induced by pollutants (Hori *et al.*, 2006). Similarly, Datta *et al.* (2007) reported that exposure of *Clarias batrachus* to arsenic induces diminution of total hepatocyte protein content.

In the current study, the exposure of *C. gariepinus* to arsenic induced marked histological changes in the liver and gills. The incidence of high DTC in the liver may be an indication of hepatic lesions and cellular damage. This result gets the support from the data recorded herein which showed elevated AST and ALT levels in plasma after arsenic exposure. Therefore, the changes in the liver histology due to arsenic reflect its high sensitivity to contaminants which could be useful as environmental monitoring (Thophon *et al.*, 2003). Also, the gills are extremely important in respiration, osmoregulation, acid base balance, and excretion of nitrogenous wastes in fish (Heath, 1995). In the present study, the gills of *C. gariepinus* bare to arsenic showed the incidence of histological alterations. This is explored by the value of DTC of exposed fish being significantly higher than those of the control fish. The gill hyperplasia, telangiectiasis and lamellar fusion were documented due to arsenic exposure, these results agree with those reported for other environmental contaminants (Van Heerden *et al.*, 2004; Abdel-Tawwab *et al.*, 2007; Simmonato *et al.*, 2008;). Therefore, arsenic causes branchial lesions in the gills *C. gariepinus* which in turn perturb gill functions. The gill hyperplasia was allied with hypoxic condition induced by metal pollution. These changes resulting from the exposure to metals could possibly due to compensatory comeback to prevent the metal entry through the gill cells (Mallat, 1985; Dangè *et al.*, 2000).

It could be fulfilled that the exposure of *C. gariepinus* to arsenic induced hurtful possessions. Alternatively the use of calcium carbonate along with arsenic (1/20 LC_{50}) abridged the harmful property. Therefore, it could be useful as a protective agent against arsenic induced injurious property.

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