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Genotoxic, Hematological and Biochemical Changes Induced by Phenol Exposure in African Catfish (*Clarias gariepinus*)

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Abstract: Phenol and phenolic compounds are xenobiotics stressful environmental factors to which animals are subjected to and have become environmental problem due to anthropogenic impact on the environment. The present study was aimed to investigate the effect of phenol toxicity on catfish (*Clarias gariepinus*) with special reference to the genotoxic, haematological and biochemical changes. For this purpose, thirty apparently normal African catfish (*Clarias gariepinus*) were divided into three equal groups as following; Group1, was not exposed to any treatment, kept in dechlorinated water and considered as control group; Group 2, was exposed to phenol at a dose level of 7mg/L water and Group 3, was exposed to phenol at a dose level of 12mg/L water for 2 successive weeks. After 2, 7, 14 days of phenol exposure, blood samples were collected for genotoxicity and hematological studies. Serum samples were collected for biochemical analysis. Tissue samples from gill arches were collected for micronucleus assay. The obtained results indicated that phenol possesses genotoxic effect as it increased the frequency of MN and NAs of catfish erythrocyte. Moreover, it induced anemia, leukopenia, oxidative damage and hepato-renal dysfunction (Increased the activities of ALT, AST, cholesterol and creatiinine levels and decreased the protein and albumin levels) especially at higher dose.

Key words: Phenol · Micronuclei · Nuclear Abnormalities · Leukopenia · Hepato-Renal Dysfunction

INTRODUCTION

Water pollution is one of the most important problems of this era, affecting human and all living organisms and deteriorating natural resources [1]. A large number of chemical compounds that get into aquatic ecosystems can cause hazardous effects on marine and freshwater organisms. Industrial effluents are indiscriminately discharged into aquatic ecosystems without any pretreatment thus creating serious problems to the non target organisms. Discharging of effluents into freshwater systems depletes the dissolved oxygen content causing heavy mortality in fish by interfering with respiratory metabolism [2]. Phenol and its compounds are ubiquitous water pollutants which come to the natural water resources from the effluents of a variety of chemical industries such as coal refinaries, phenol manufacturing, pharmaceuticals and industries of resin, paint, dyeing, textile, leather, petrochemical and pulpmill [3]. Relatively high concentrations of phenol are found in rivers near the outlets of channels where industrial wastewaters have been discharged [4]. Moreover, natural processes such as the decomposition of plant matter also contribute to phenol accumulations in the aquatic environment [5]. Phenol pollution presents a threat against natural environment and human health [6]. Despite the active metabolism and detoxification of phenol in fish, sub-lethal doses of phenol interfere with various enzyme activities and can produce many unpredictable changes in fish. It has been found that phenol, after entering into the fish body, affects the metabolism, survival, growth [7] and reproductive potential of fish [8]. Moreover, phenol and its derivatives have toxic effects for fish; they induce carcinogenic, immunotoxic and physiological effects and have a bioaccumulation rate along the food chain due to its lipophilicity [9,10,11]. The information about the phenol toxicity at ecosystem level is limited. Therefore, there is a need to investigate the phenol toxicity to fish and aquatic ecosystems in detail. The present study aimed to investigate the impact of phenol toxicity on catfish (*Clarias gariepinus*) with special reference to the genotoxic, haematological and biochemical changes.

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MATERIALS AND METHODS

Experimental Fish: Thirty apparently normal African catfish (*Clarias gariepinus*) with an average body weight of 70±5g obtained from a semi-intensive aquaculture facility in the fish research station (World Fish Center, Abbassa, Egypt) were used in the present experiment. Fish were acclimated for a period of 7 days in the laboratory condition prior to the experiment. They were fed twice a day with a balanced commercial fish pelleted diet, kept in aquaria (40 × 80 × 30 cm) containing dechlorinated tap water and oxygen supply was maintained in using an electric aerator pumps.

Phenol: Technical grade of phenol (C_6 H_5 OH), M.W. 94.11 with freezing point of 39.5-41.0°C was obtained from El–Nasr Pharmaceutical Chemical Company. The stock solution was prepared by dissolving phenol in distilled water (solvent).

Experimental Design: Fish were divided into three equal groups as following; Group1, fish in this group were not exposed to any treatment, kept in dechlorinated water and considered a control group; Group 2, was exposed to phenol at a dose level of 7mg/L water and Group 3, was exposed to phenol at a dose level of 12mg/L water for 2 successive weeks [12]. The water was renewed every 3 days followed by addition of tested pollutant (Phenol) as phenol is lost by volatilization [6].

Samples: Blood samples were collected from fish caudal vein of each group after 2, 7, 14 days of phenol exposure and were divided into three parts. The first one was collected into clean dry tube containing 10% disodium salt of ethylenediamine tetra-acetic acid and used for hematological and genotoxic studies. The second sample was collected into plain centrifuge tube for serum preparation and used for biochemical studies. The third part was collected in sodium citrate containing tube and its plasma was taken for analysis antioxidant enzyme (G6PDH). Tissue samples from gill arches were collected for micronucleus assay.

Genotoxicity Studies

Measurement of Micronucleus (MN) and Nuclear Abnormalities (NAs): Peripheral blood samples were obtained from the caudal vein of the specimens on (2d, 7d, 14d) and smeared on clean slides. After fixation in pure ethanol for 20 min, slides were left to air-dry and then the smears were stained with 10% Giemsa solution for 25 min. Erythrocyte micronuclei according to Al-Sabti and Metcalfe [13] and nuclear abnormalities NAs after Carrasco *et al*. [14] were recorded. From each slide, 1000 cells were scored for frequencies of MN and NAs. The MN test on gill cells was performed according to Cavas and Ergene-Gozukara [15]. Cells from gill arches were isolated and fixed, smeared onto clean slides and stained with 5% Giemsa solution for 30 min.

Hematological Studies: Hematological parameters including erythrocyte count (RBCs), packed cell volume (PCV), hemoglobin concentration (Hb), total leucocyte count (TLC) and differential leucocytic count (DLC) were done according to Feldman *et al.* [16].

Biochemical Studies: Serum samples were used for determination of the following parameters; serum glucose was performed according to Trinder [17]. The activities of alanine (ALT) and aspartate (AST) amino transferases were performed according to Reitman and Frankel [18]. Cholesterol was assayed according to Allain *et al.* [19] and serum creatinine was performed after Fabiny and Eringhausen [20]. Serum total proteins were determined according to Weicheselbaun [21], serum albumin was assayed after Dumas and Biggs [22] and serum globulins was calculated by subtracting the obtained values of albumin from values of total proteins. The activity of antioxidant enzymes glucose-6-phosphate dehydrogenase (G6PDH) was done on plasma samples according to Kornberg [23]. The above mentioned biochemical parameters were assayed using commercial Biodiagnostic reagent kits.

Statistical Analysis: The data were given as individual values and as mean ± standard deviation. Comparisons between the means of various groups were analyzed using one way ANOVA as described by Snedecor and Cochran [24].

RESULTS AND DISCUSSION

Results of Micronucleus Assay: The frequency of micronuclei in erythrocyte and gill cells of phenol exposed fish and control fish are summarized in Table 1 and Figure 1.

The obtained data revealed a significant increase in the frequencies of erythrocyte and gills micronuclei of catfish exposed to higher dose of phenol (Group3) 48hrs post phenol exposure till the end of the experimental period. Whereas, catfish exposed to lower dose of phenol

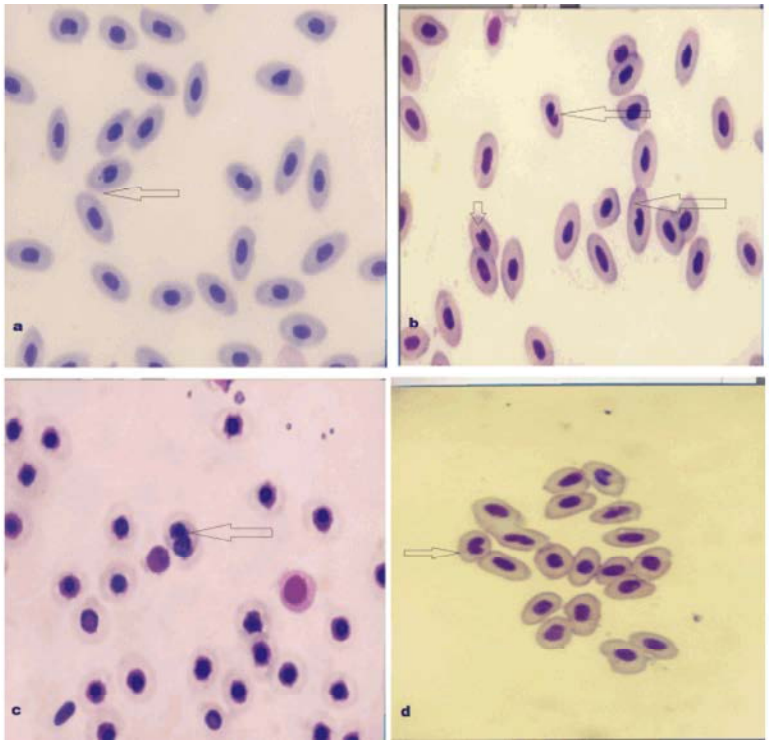

Fig. 1: Erythrocytes of African catfish (*Clarias gariepinus*) with (a) Micronuclei (MN), nuclear abnormalities: (b) Blebbed nucleus (BL) (small arrow) and Lobed nucleus (LB) (large arrows), (c) Binuclei (BN) and (d) Notched nucleus (NT)

Table 1: Frequencies of of micronuclei (MN) in erythrocytes and gill of catfish exposed to different doses of phenol

	Parameters		
Time	Groups	Micronucleus in blood	Micronucleus in gill
2d	Group (1)	1.67 ± 0.58^{b}	0.33 ± 0.47^{b}
	Group (2)	2.33 ± 0.58^{b}	0.63 ± 0.48^{ab}
	Group (3)	4.00 ± 1.00^{a}	1.19 ± 0.55^{a}
7d	Group (1)	2.00 ± 1.00^{b}	0.61 ± 0.48^{b}
	Group (2)	2.33 ± 0.58^{b}	1.16 ± 0.59^{ab}
	Group (3)	5.67 ± 0.58^{a}	1.50 ± 0.58^{a}
14d	Group (1)	1.33 ± 0.58^{c}	0.60 ± 0.49^{b}
	Group (2)	3.33 ± 0.58^{b}	1.66 ± 0.61^{a}
	Group (3)	6.00 ± 1.00^{a}	1.78 ± 0.93^{a}

Values represent means ± SD

Means with different superscripts (a, b, c) within the same column are significantly different at P value $P < 0.05$.

(Group2) showed significant elevation in erythrocyte and gills MN frequencies on the 2nd week post exposure compared to control group. It has been suggested that a variety of biomarkers and bioassays in the laboratory and field studies have been used in determining the genotoxic effect of pollutants. The micronucleus assay is the most widely applied method because of its simplicity, reliability, sensitivity and proven suitability for fish species [25]. Micronuclei are cytoplasmic chromatin-containing bodies formed when acentric chromosome fragments or chromosomes lag during anaphase and fail to become incorporated into daughter cell nuclei during cell division. The obtained results reflect the genetic damage induced by phenol [26]. Its potency in disruption of the cytoplasmic microtubules complex, mitotic spindle and induction of micronuclei has been demonstrated in mussels [27]. Our results are in accordance with Farah *et al.* [28] who found significant increase in the micronuclei frequency of fish (*Channa punctatus*) 72 and 96 hours post pentachlorophenol exposure.

Table 2: Frequencies of nuclear abnormalities (NA) in erythrocytes of catfish exposed to different doses of phenol

		Nuclear abnormalities				
Time	Groups	Blebbed	Lobed	Binucleated	Notched	Total
2d	Group (1)	0.10 ± 0.00^{a}	0.07 ± 0.06^{a}	0.10 ± 0.00^{b}	0.13 ± 0.06^{a}	0.40 ± 0.10^{b}
	Group (2)	0.17 ± 0.06^{a}	0.13 ± 0.06^{a}	0.10 ± 0.00^{b}	0.17 ± 0.06^{a}	0.57 ± 0.06^{ab}
	Group (3)	0.13 ± 0.06^{a}	0.20 ± 0.10^{a}	0.17 ± 0.06^{a}	0.20 ± 0.10^{a}	0.70 ± 0.17^{a}
7d	Group (1)	0.07 ± 0.06^{b}	0.07 ± 0.06^{a}	0.10 ± 0.00^{b}	0.07 ± 0.06^{a}	0.30 ± 0.10^{b}
	Group (2)	0.13 ± 0.06^{b}	0.07 ± 0.06^{a}	0.13 ± 0.06^{b}	0.10 ± 0.00^{ab}	0.43 ± 0.06^{b}
	Group (3)	0.23 ± 0.06^{a}	0.13 ± 0.06^{a}	0.23 ± 0.06^{a}	0.17 ± 0.06^{b}	0.76 ± 0.12^{a}
14d	Group (1)	0.03 ± 0.06^{b}	0.07 ± 0.06^{b}	0.07 ± 0.06^{a}	0.10 ± 0.00^{b}	0.27 ± 0.06^{b}
	Group (2)	0.13 ± 0.06^{a}	0.10 ± 0.00^{b}	0.13 ± 0.06^{a}	0.20 ± 0.10^{a}	0.56 ± 0.15^{a}
	Group (3)	0.20 ± 0.10^{a}	0.27 ± 0.15^{a}	0.17 ± 0.06^{a}	0.23 ± 0.06^{a}	0.87 ± 0.15^{a}

Values represent means ± SD

Means with different superscripts (a, b, c) within the same column are significantly different at P value P <0.05.

Table 3: Effect of phenol exposure on erythrogram of African Catfish (*Clarias gariepinus*)

		Parameters				
Time	Groups	RBCs ($x10^6$ /µl)	PCV (%)	Hb (g/dL)	MCV (fl)	MCHC (%)
2d	Group (1)	2.63 ± 0.32^{a}	38.00 ± 1.00^{a}	10.40 ± 0.60^{a}	145.61 ± 16.43^{a}	27.37 ± 1.37^{a}
	Group (2)	2.80 ± 0.35^{a}	39.00 ± 1.00^{a}	10.67 ± 0.61^{a}	140.83 ± 19.06^{a}	27.39 ± 2.25^{a}
	Group (3)	3.00 ± 0.20^{a}	38.67 ± 0.58^{a}	11.20 ± 0.98^{a}	130.33 ± 9.24^{a}	29.10 ± 2.38^{a}
7d	Group (1)	2.67 ± 0.42^{a}	37.00 ± 1.00^{a}	10.60 ± 0.53^{a}	138.75 ± 20.64^{b}	28.68 ± 2.05^{a}
	Group (2)	2.30 ± 0.20^{ab}	36.67 ± 1.53^{a}	9.93 ± 0.50^{a}	159.42 ± 17.94^{a}	27.09 ± 2.49^{ab}
	Group (3)	1.91 ± 0.20^{b}	30.67 ± 1.53^{b}	7.46 ± 0.45^{b}	160.28 ± 25.15^{a}	24.31 ± 0.80^{b}
14d	Group (1)	2.46 ± 0.27^{b}	36.33 ± 1.53^{b}	10.20 ± 0.31^{b}	148.90 ± 19.52^{b}	28.09 ± 0.96^{a}
	Group (2)	3.30 ± 0.20^{a}	40.00 ± 1.00^{a}	11.47 ± 0.42^{a}	121.21 ± 9.24^{c}	28.67 ± 0.41^{a}
	Group (3)	1.51 ± 0.08^{c}	29.00 ± 1.00^{c}	6.47 ± 0.42^{c}	191.85 ± 8.27^{a}	22.30 ± 2.03^{b}

Values represent means ± SD

Means with different superscripts (a, b, c) within the same column are significantly different at P value P <0.05.

Results of Nuclear Abnormalities: Nuclear abnormalities (NAs) of different experimental groups are shown in Table 2 and Figure 1.

Our results revealed significant increase in the frequency of total nuclear abnormalities of catfish exposed to higher dose of phenol (Group3) 48hr. post exposure while marked increase in the frequency of total nuclear abnormalities was recorded on the 2nd week in catfish exposed to lower dose of phenol (Group2) in comparable to control group. The recorded nuclear abnormalities were in the form of blebbed (BL), lobed (LB), binucleated (BN) and notched nucleus (NT) (Fig. 1). Blebbed nucleus (BL) presented a relatively small evagination of the nuclear membrane, which contained euchromatin. Evaginations larger than the BL, which could have several lobes, were classified as lobed nucleus (LB). Cells with two nuclei were considered as binucleates (BN) and nuclei with depth into a nucleus were recorded as (NT). The mechanisms underlying the formation of NAs have not been fully explained, several studies indicated that NAs are induced in response to exposure to genotoxic agents [29,30]. The obtained results are in agreement with Cavas and Ergene-Gozukara [15] who demonstrated Nas in erythrocytes of O. niloticus exposed to a textile mill effluent. Baršienë *et al*. [31] also recorded nuclear abnormalities in mature erythrocytes of peripheral blood and immature erythrocytes of cephalic kidney in turbot (Scophthalmus maximus) and in the Atlantic cod exposed to 0.5 ppm of spiked crude oil.

Clinicopathological Findings:

Erythrogram: Mean values of erythrogram [packed cell volume (PCV %), hemoglobin concentration (Hb), erythrocytes count (RBCs), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC)] of phenol intoxicated catfish (clarias gariepinus) are illustrated in Table 3.

Erythrogram mean values showed a significant increase in RBCs count, PCV and Hb concentration in catfish exposed to lower dose of phenol on the 2nd week post exposure compared to control group. The obtained data may be referred to the hypoxic stress and stimulation of erythropoietin by elevated demands for O_2 as a result of increased metabolic activity or destruction of gill membranes by phenol [2, 32]. However, significant

Table 4: Effect of phenol exposure on leukogram of African Catfish (*Clarias gariepinus*)

		Parameters				
Time	Groups	TLC ($\times10^3$/µl)	Heter. ($\times10^3$/µl)	Lymph. ($\times10^3$/µl)	Monocyte ($\times10^3$/µl)	Esino. ($\times10^3$/µl)
2d	Group (1)	15.21 ± 0.40^b	3.57 ± 0.40^b	11.29 ± 0.30^a	0.31 ± 0.03^b	0.32 ± 0.05^a
	Group (2)	18.20 ± 1.21^a	8.87 ± 0.80^a	8.07 ± 1.30^b	0.56 ± 0.02^a	0.44 ± 0.02^a
	Group (3)	17.43 ± 0.60^a	8.67 ± 1.62^a	7.53 ± 1.03^b	0.59 ± 0.05^a	0.47 ± 0.06^a
7d	Group (1)	15.60 ± 0.63^b	3.74 ± 0.34^b	10.80 ± 1.10^a	0.49 ± 0.03^b	0.15 ± 0.02^a
	Group (2)	17.20 ± 1.22^a	10.97 ± 0.45^a	5.60 ± 0.60^c	0.87 ± 0.03^a	0.19 ± 0.03^a
	Group (3)	13.30 ± 1.10^c	4.07 ± 1.62^b	7.90 ± 0.46^b	0.29 ± 0.04^c	0.16 ± 0.03^a
14d	Group (1)	16.40 ± 0.80^b	4.00 ± 0.20^b	11.93 ± 0.50^a	0.51 ± 0.04^b	0.18 ± 0.03^a
	Group (2)	19.27 ± 0.70^a	12.00 ± 0.60^a	6.07 ± 0.31^b	0.76 ± 0.03^a	0.20 ± 0.02^a
	Group (3)	10.67 ± 0.83^c	3.63 ± 0.35^b	6.53 ± 0.42^b	0.31 ± 0.03^c	0.23 ± 0.03^a

Values represent means ± SD

Means with different superscripts (a, b, c) within the same column are significantly different at P value P <0.05.

reduction in the previous parameters was recorded in catfish exposed to higher dose of phenol on the 1st and 2nd week post exposure compared to control group. The reduction in hematological values with increased MCV and decreased MCHC denotes the developing of macrocytic hypochromic anemia. This anemia may be due to erythropoiesis and osmoregulatory dysfunction or due to increased rate of erythrocytes destruction in haematopoietic organs by phenol [33]. Phenolic compounds are frequently considered as reactive oxygen species generating agents that lead to major cell damage and oxidation of membrane polyunsaturated lipids [34] and erythrocyte destruction. Our results are in accordance with Chen [35] and Ali and Amer [36].

Leukogram: Means values of leukogram (total leukocytic count, heterophil, lymphocyte, monocyte and esinophil count) of different experimental groups are illustrated in Table 4.

Compared to the control group, leukocytosis with heterophilia, monocytosis and lymphopenia were showed in group 2 all over the experimental period and 2d. post phenol exposure in group 3 which exposed to higher dose. It could be interpreted as stress response following increased corticosteroid level. Corticosteroid decrease stickiness and margination of heterophil resulting in their retention in circulation. Moreover, corticosteroid induces lymphocyte apoptosis and altering their patterns of recirculation causing lymphopenia [37]. In addition, Gabriel *et al*. [38] stated that the increase in WBC (Leukocytosis) may have resulted from the excitation of defense mechanism of the fish to counter the effect of the toxicant. On the other hand significant leukopenia associated with lymphopenia and monocytopenia was recorded in catfish exposed to higher dose of phenol on the 1st and 2ndweek post exposure. The recorded leukopenia reflects the adverse effect of phenol on leukocyte which may be induced by primary or secondary changes in haematopoietic organs [39]. The obtained results are in agreement with previous results of Maule *et al*. [40] and Mekkawy *et al*. [41].

Biochemical Evaluation: Statistical analysis of different biochemical parameters of experimental groups is illustrated in Tables 5 & 6.

Significant hyperglycemia was recorded in phenol exposed groups (2&3), 48 hrs after exposure till the end of experimental period compared to the control group. The blood sugar level represents a dynamic balance between the rate at which the sugar is entering the blood from the liver and the rate at which it is being removed by the body tissue from the blood. The observed hyperglycemia in phenol exposed groups may be due to increased glucocorticoid secretion associated with stressful conditions resulted from phenol exposure [42]. Similar increase in whole blood glucose concentrations were reported in fish exposed to 3.2, 7.3 and 8.5 mg l-1 phenol [43].

Non significant increase was recorded in the activity of glucose-6-phosphate dehydrogenase (G6PDH) in catfish exposed to lower dose of phenol (Group2). G6PDH has long been recognized as an antioxidant enzyme [44] and as a biomarker of pollution in fish [45]. The non significant increase in G6PDH activity may represents protection against elevated levels of reactive oxygen species in cells exposed to an oxidative stress through the increased NADH production that in turn maintains the level of glutathione in cells and protect it from oxidative damage [46]. On the other hand, significant decrease was recorded in G6PDH activity of catfish exposed to higher dose of phenol on the 2nd week post exposure. The decline in G6PDH activity reflects the increased oxidative damage induced by the higher dose of phenol. The obtained results are in parallel with Ashwani [34].

Table 5: Effect of phenol exposure on some biochemical parameters of African Catfish (*Clarias gariepinus*)

		Parameters					
Time	Groups	Glucose (mg/dl)	G6PDH (mu/ml)	ALT (IU/L)	AST (IU/L)	Chol. (mg/dl)	Creatinine (mg/dl)
2d	Group (1)	97.83 ± 2.93^{c}	60.47 ± 2.32^{a}	10.17 ± 2.02^{a}	17.80 ± 2.55 ^{ab}	286.27 ± 6.11^{a}	0.31 ± 0.03^{a}
	Group (2)	116.17 ± 14.63^{b}	61.93 ± 2.47^{a}	9.00 ± 1.11^{a}	15.60 ± 1.25^{b}	289.40 ± 5.33^{a}	0.30 ± 0.02^{a}
	Group (3)	136.00 ± 3.51^{a}	58.53 ± 3.83^{a}	10.60 ± 0.85^{a}	20.40 ± 2.30^{a}	288.00 ± 3.06^{a}	0.33 ± 0.04^{a}
7d	Group (1)	87.80 ± 5.70^{c}	61.13 ± 2.42^{a}	12.60 ± 0.53^{b}	15.57 ± 1.40^{b}	288.00 ± 5.29^{b}	0.52 ± 0.03^{b}
	Group (2)	100.67 ± 4.04^{b}	62.47 ± 3.83^{a}	14.83 ± 2.26^{b}	18.27 ± 2.30^{b}	291.93 ± 4.43^{b}	0.62 ± 0.04^{b}
	Group (3)	127.00 ± 4.04^{a}	63.93 ± 4.84^{a}	38.80 ± 1.17^{a}	39.00 ± 15.39^{a}	549.60 ± 1.83^{a}	1.00 ± 0.22^{a}
14d	Group (1)	90.13 ± 2.20^{c}	56.27 ± 1.62^{a}	14.40 ± 1.25^{c}	23.44 ± 1.99^{c}	296.90 ± 2.76^{c}	0.56 ± 0.03^{c}
	Group (2)	119.67 ± 8.50^{b}	59.67 ± 5.69^{a}	29.93 ± 1.60^{b}	35.53 ± 2.86^{b}	386.83 ± 4.25^{b}	1.00 ± 0.09^{b}
	Group (3)	147.00 ± 5.57^{a}	51.47 ± 3.00^{b}	40.00 ± 1.53^{a}	67.83 ± 4.51^{a}	610.20 ± 5.57^{a}	1.30 ± 0.07^{a}

Values represent means ± SD

Means with different superscripts (a, b, c) within the same column are significantly different at P value P <0.05.

Table 6: Effect of phenol exposure on protein profile African Catfish (*Clarias gariepinus*)

		Parameters			
Time	Groups	*T. Proteins* (g/dl)	Albumin (g/dl)	Globulins (g/dl)	A/G Ratio
2d	Group (1)	4.77 ± 0.25^{a}	3.07 ± 0.12^{a}	1.70 ± 0.17^{a}	1.81 ± 0.17^{a}
	Group (2)	5.15 ± 0.59^{a}	2.89 ± 0.37^{a}	2.26 ± 0.35^{a}	1.29 ± 0.21^{a}
	Group (3)	4.60 ± 0.35^{a}	2.40 ± 0.40^{a}	2.20 ± 0.35^{a}	1.09 ± 0.39^{a}
7d	Group (1)	4.57 ± 0.68^{a}	2.73 ± 0.28^{a}	1.84 ± 0.45^{a}	1.52 ± 0.24^{a}
	Group (2)	4.70 ± 0.56^{a}	2.67 ± 0.34^{a}	2.03 ± 0.81^{a}	1.58 ± 1.00^{a}
	Group (3)	2.70 ± 0.45^{b}	1.30 ± 0.37^{b}	1.40 ± 0.30^{a}	0.93 ± 0.50^{a}
14d	Group (1)	5.15 ± 0.44^{a}	3.57 ± 0.11^{a}	1.62 ± 0.42^{a}	2.28 ± 0.47^{a}
	Group (2)	3.85 ± 0.57^{b}	2.14 ± 0.41^{b}	1.71 ± 0.18^{a}	1.25 ± 0.15^{b}
	Group (3)	2.66 ± 0.27^{c}	1.48 ± 0.33^{b}	1.18 ± 0.24^{a}	1.25 ± 0.40^{b}

Values represent means ± SD

Means with different superscripts (a, b, c) within the same column are significantly different at P value P <0.05.

A marked increase in serum transferases activities (ALT and AST) were observed in groups 3&2 on the 1st and 2nd week post phenol exposure, respectively in comparison to control group. The pronounced increases in transaminases activities reflect the hepatic injury induced by phenol exposure. Phenol has lipophylic nature, it accumulated on the hydrophilic lipid bilayer of biological membranes, generates free radicals and increase lipid peroxidation of the liver that resulting in hepatic damage. Similar results were obtained by Ashwani, [34] and Owen and Rosso [47].

Compared to control group, hypercholesterolemia was recorded in phenol exposed fish (Group 2&3) on the 2nd and 1st week, respectively. The observed increase in serum total cholesterol may be due to release of cholesterol and other lipids constituents from damaged cell membranes and decrease hepatic excretion of cholesterol due to phenol exposure [48].

Significant increase in creatinine levels was showed in phenol exposed fish (Group 2&3) on the 2nd and 1st week, respectively. Creatinine elevation reflects kidney damage as it is mainly excreted through kidney in most fish [49]. Similar results are obtained by Alaa *et al.* [50] who recorded significant increase in serum creatinine level exposed to sublethal doses of 4-nonylphenol (0.05, 0.08 and 0.1 mgl^{-1}) of the African catfish (*Clarias gariepinus*).

Concerning serum proteins, statistical analysis of our data revealed a significant hypoproteinemia with hypoalbuminemia in Groups 2&3 on the 2 nd and 1st week, respectively compared to control group. Under conditions of stress, many organisms mobilize proteins as an energy source via oxidation of amino acids. So the recorded hypoproteinemia in the present study may be attributed to stress mediated immobilization of these compounds to fulfill an increased element for energy by fish to cope with environmental stress induced by phenol exposure [6]. This phenomenon is previously recorded for different fish species subjected to phenol [51]. Furthermore, the depletion of protein in our study reflects both hepatic and renal damage induced by phenol [52].

The achieved results clarified the adverse effects of phenol on catfish. It possesses a genotoxic effect as it increased the frequency of MN and NAs of erythrocyte catfish. It induced anemia, leucopenia and hepato-renal dysfunction especially at higher doses.

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