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Effect of 4-Nonylphenol on Some Biochemical Parameters and Histological Changes in Liver of Toad Arab *Bufo arabicus* in Taif City, Saudi Arabia

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Abstract: Ecosystem imbalance due to the spread of various types of pollutants and their adverse effects, therefore, this study aimed to identify the impact of contaminated environmental fourth nonylphenol phenol (4-Nonylphenol) on Toad Arab *Bufo arabicus*biochemical aspects and histological changes. Methods The toads took from the seesd region to the lab in environmental similar to natural environmental conditions. LC_{50} value (4, 8 µ/l) was calculated and then the toad divided into three groups. Acute toads group received 2.4 µg/l for four days, chronic toads group received 0.48 µg/l for a month and third group served as control toads. The results showed significant decrease in RBCs count, the hemoglobin content and the value of hematocrit. In return, increase in WBCs number, the mean corpuscular volume and mean corpuscular hemoglobin was pointed in toad exposed to acute and chronic concentrations compared to control group. Noticeable change in mean corpuscular hemoglobin concentration was indicated in toads exposed to both concentration of 4-Nonylphenol. A significant increase in the serum sugar level and ALT, AST, ALP enzymes which were time and dose depending of both treated groups. Liver histological examination pointed that acute and chronic doses caused rupture the endothelial lining the blood vessels as well as vascular congestion, blood sinusoid widening with lymphocytes infiltration. It is concluded that 4-Nonylphenol induced histological distortions and efficiency of liver enzymes of Toad Arab *Bufo arabicus*. Therefore it might provide a novel strategy target in management of protecting this species fromextinction.

Key words: 4-Nonylphenol · L-Hemoglobin · Liver Enzymes · Liver Tissue · Toads

INTRODUCTION

The most common use of 4-Nonylphenol is in the production of plastics, detergents and pesticides which are usually thrown out as waste in the sewage [1]. Talmage [1] and Fay *et al*. [2] confirmed that contaminated environmental 4-Nonylphenol and ethoxylatesnonyl is used worldwide in the production of plastics, pesticides and cleaning products and present in sewage and in cosmetics products [3]. The phenols including 4-Nonylphenol is considered of most important phenols environmental pollutants and more dangerous, as a result of its widespread intensive use, it found in rivers, valleys, groundwater and industrial waste water and sewage processor water and raise to the extent that prevents the natural degradation and remains and accumulates in the environment, causing a lot of damage and organisms disease including man [4].Amphibians provide extremely visible indicator of pollution, they may be sensitive to global environmental changes. Therefore, the amphibians can be considered as an early indicator of a "global environmental disaster" [5]. A direct effect of the environmental pollutant Nonylphenol and its derivatives on sex hormones in vertebrates was existence [6-9]. Shannon and Veeramachaneni [10] indicated that exposure to low concentrations of the 4-Nonylphenol weakens the sperm fertility in frog *Xenopuslaevis*. Several studies suggest that chemical contaminant have effects on adults' amphibians and reportedly induces abnormalities and deformities [11-13]. Whereas, Gürkan

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and Hayretdag, [14] reported that copper sulfate has a negative morphological and histological on the growth and development of the green toad *Bufoviridis*. However, Hinton and Lauren [15] proved that, the liver is important in performs many function, including metabolic conversion and carbohydrates stored and any changes in the liver structure gives the biological markers indicate to exposure of the organism to stress of various environmental pollutants including the 4-Nonylphenol.Coppo *et al*. [16] reported the physiological variation of enzymatic activities in the blood of bull frog, *Ranacatesbeina*. Blood and urine physiological values in the captive frog, *Ranacatesbeina*have also been reported by Coppo*et al.* [17]. In light of the above and the lack of information about the toxicity ecosystems of 4- Nonylphenol on amphibians biochemical constituents and liver tissue. This research has been done on Toad Arab *Bufo arabicus* which on the IUCN red list of threatened species and live in all environments in Saudi Arabia where the availability of water [18, 20].

MATERIALS AND METHODS

The Study Area: The study was conducted on the summer season 1432/1433 in seesd region in Taif city, which is located north-east of Taif along the Wag valley to the EL-Arg Valley through several neighborhoods with high population density. The study area is characterized by a continuously stream draws(near the water treatment plant) its water from different sources and there is many farms of the vegetables and fruit on the outskirts of stream. Samples of water stream in the study area were collected in clean glass bottles for chemical analysis to determine the most abundant heavy or toxic metalsaccording [19]. Wateranalyzed using spectrophotometer (DR252499, USA).

Experimental Animals

Arabian Algome: The common name: Arabian Toad and The scientific name: *Bufo arabicus.* The toads in the study area are found within the waterway and sometimes on the edges and in places muddy wet [20].

Nonylphenol Phenol: The 4- Nonylphenolwas purchased from Bayouni Trading Company Ltd. Jeddah/ KSA. Colorless, viscous liquid with a smell similar to phenol. Boiling Point is 293-2975, a slow solubility, chemical formula C15H24O, molecular weight is 220 0.35, with 109 Pa (vapor pressure) and the figure qualitative/density is 9450 g/cm^3.Two selected Concentration are used in this work, acute concentration (half lethal dose) of 2.4 micrograms / liter for four days and chronic concentration (one over ten of lethal dose) 0.48 micrograms / liter for a month, according to Litchield and Wilcoxon[21] method.

Toad Anatomy: At the end of the study (30 days), all toads set were sacrificed under chloroform anesthesia. The thorax was dissected, the liver were excised immediately and cut into two small pieces and processed forlight microscope examination as follows:

The tissues were fixed in 10% buffered formalin and processed as usual to prepare 5-µm- thick paraffin sections. The liver paraffin sections were stained with hematoxlin and eosin (H&E), [22].

Blood Samples: Blood samples were collected from toads by medical needle size 50.0 mm through withdrawal of blood from the heart directly. After the withdrawal of blood from the toads were collected in test tubes containing heparin for measuring blood components and pipelines without heparin to conduct chemical analysis of blood samples and then numbering the samples. A centrifuge (Thermo IEC) was used for serum in the pipeline that does not contain heparin and to conduct chemical analyzes as follows:

Blood Parameters: Red blood cells (RBCs), White blood cells (WBC), Hemoglobin (Hb), Hematocrit (Ht), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), were determined according to Dacieand Lewis method [23].

Blood Biochemical Assay: Glucose, Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline phosphatase (ALP), were determined by kits of SGMitalia Company [24].

Statistical Analysis: Statistical analysis was conducted with SPSS, V.18. Data are expressed as mean ± S.D., The difference among means has been analyzed by one way ANOVA followed by student *t* test and probability value of $P < 0.01$ was considered to be statistically significant.

RESULTS

LC_{50} of (4-Nonyl Phenol): After exposing the animal experiments Toad Arab ((*Bufo arabicus*) of contaminated environmental 4-nonylphenol found that the value of the LC_{50} (96-hr) equal to 4, 8µg / l. Tables (1, 2) clarified that

Table 1: Half lethal concentration (96hrs LC_{50}) of (4-Nonyl phenol) in adult toad

4- Nonylphenol concentrations (µg/l)	Number of Toads	Number of dead toads (Mortalities)	A	B	A X b
0	30	0	0	0	0
2	30	3	2	105	3
4	30	9	2	6	12
6	30	21	2	15	30
8	30	30	2	25, 5	51

Σ a X b = 96

Σ a X b

Half lethal concentration of LC_{50} =Highest dose at 96 hr

LC_{50} of (4-Nonyl phenol)= 8

N= 30

LC_{50} of (4-Nonyl phenol)= 8 – 3, 2= 4, 8 µg/l

A= Differences in concentrations between groups.

B= Mean value of dead toads between each two successive groups.

N= Number of toads in each group.

Table 2: Ilustrates the elements that have been estimated in the seesd water stream

No	Name of test	Sample	Maximum permissible level
1	Ammonia as nitrogen(NH_3-N)	0.69	5mg/l
2	Nitrate as nitrogen(NO_3-N)	65.3	10mg/l
3	TDS	776	0
4	PH	7.0	7.0 – 9.0
5	Suspended solids	292	5 mg/l
6	Free chlorine	0	0.5mg/l
7	Turbidity	167	10mg/l
8	Aluminum (Al)	Nil	5mg/l
9	Copper(Cu)	0.39	0.2mg/l
10	Chromium(Cr)	0.13	0.01mg/l
11	Boron(B)	1.5	0.75mg/l
12	Lead(Pb)	Nil	5mg/l
13	Manganese(Mn)	0.2	0.2mg/l
14	Molybdenum(Mo)	2.1	0
15	Cobalt(Co)	0.02	0.05mg/l
16	Nickel(Ni)	0.02	0.02mg/l
17	Selenium (Se)	0.09	0.02mg/l
18	Phenol(P)	0.001	0.002mg/l
19	Iron(Fe)	0.02	5mg/l
20	Zinc (Zn)	0.17	2mg/l

contaminated environmental 4-Nonylphenol is more toxic than other pollutants as the value Microgram / L and the elementsvalues that have been estimated in the seesd water stream were illustrated in Table (2).

Blood Measurements: It clear in Table (3) a significant ($p<0.01$) decrease in the number of RBCs, hemoglobin content, the hematocrit value in toads exposed to the impact of the acute (2.4µg \ l) and chronic (0.48 µg/ l) of 4- Nonylphenolcompared to the control value (F-value = 25, 118 and 66 respectively). The statistical analyzes revealed a significant increase in the MCV and MCH values in toads exposed to acute and chronic concentration of 4- Nonylphenol, while the change in value of the MCHC found is noticeable in toads exposed to chronic concentration and markedly for the toads exposed to acute concentration compared to the control group. However, when comparing the number of WBCs of the toads in the all groups .Table (3) showed increase in the number of white blood cells at both acute and chronic concentrations compared to control group (F-value = 62).

Blood Sugar and Liver Functions: Toads exposed to acute and chronic concentration of 4-Nonylphenol led to a marked increase in the level of blood sugar and activity of AST, ALT and ALP enzymes compared to the control group with significant difference (F-value = 63, 31, 6, 35 respectively) at $p <0.1$, Table (4).

Table 3: Blood parameters of *Bufo arabicus*exposed to acute and chronic concentrations of 4–Nonyl–Phenol .

Treatments	RBCs (X 10^3/mm^3)	Hb (g/100 ml)	Ht (%)	MCV (μ m^3/cell)	MCH Pg/cell))	MCHC (g/100ml)	WBC_s (X10^3/mm^3)
Group I Control group (Toads reared in dechlorinated Tap water during the experimental periods)	0.47±0.038 A	5.88±0.13A	26.80.56±A	595± 46B	131±10B	21.95± 0.09B	16±0.85 C
Group II Acute exposure (Toads exposed to 2.4 \l of 4-Nonyl-phenol for 96 hrs.)	0.275± 0.03B	5.13±0.06B	21.0±0.53B	819±72A	201± 19A	24.5± 0.4A	28.0±1.6B
Group III Chronic exposure (Toads exposed to 0.5 \l of 4-Nonyl-phenol for 30 days)	0.17±0.02C	3.53±0.12C	17.0±0.7C	1075±105A	227± 28A	20.8± 0.55B	35.0±1.16A
F-Values	25**	118**	66**	9**	6**	23**	62**

Data are represented as means of six samples ± S.E.

Means within the same column, with the same letter for each parameter are not significantly different, otherwise They do (SAS, 2000) ** Highly significant difference at $P < 0.01$

Table 4: Serum constituents of *Bufo arabicus* exposed to acute and chronic concentrations of 4–Nonyl–Phenol

Treatments	Glucose (mg/100ml)	AST (u/l)	ALT (u/l)	ALP (u/l)
Group I Control group (Toads reared in dechlorinated Tap water during the experimental periods)	18.0±0.17C	24.8±1.35C	16.8±0.41B	39.0±4.37C
Group II Acute exposure (Toads exposed to 2.4 \1 of 4-Nonyl-phenol for 96 hrs)	59.3± 5.7B	95.0±1.89A	20.25±2.19A\B	161±13.6A
Group III Chronic exposure (Toads exposed to 0.5 \1 of 4-Nonyl-Phenol for 30 days)	113±4.15A	71.5±10.9B	23.8±1.08A	120±11.04B
F-Values	63**	31**	6**	35**

Data are represented as means of six samples ± S.E.

Means within the same column, with the same letter for each parameter are not significantly different, otherwise They do (SAS, 2000) ** Highly significant difference at$P< 0.01$

Histological Changes in Liver: Histological examination of the normal liver tissue of the toad it consists of several lobules, each lobule composed of plates of hepatic cells radiated from central veins and surrounded by blood sinusoid and granular pigment cells. Hepatocytes contain large nuclei and eosinic cytoplasm. Central veins round while the portal veins are compared larger in size and looks either empty or contains few blood cells and the bile duct seem rounder and is surrounded by a layer of cubic cells of (Fig. 1a). 4-Nonylphenol treated toad trab*Bufo arabicus* had damaged hepatic tissues. Accordingly, the hepatocytes of the frog liver treatment with acute dose (2.4μg/l) congestion of the central vein with red blood cells, hepatocytes necrosis as well as the increased of melanomacrophage cells (Fig. 1b), widening in the blood sinusoids (Fig. 1c), incidence of lymphocytes and neutrophils infiltration (Fig. 1d). However, examination of the liver tissue exposed to chronic treatment (0.48μg/l) showed rupture and separation of the epithelial lining the central vein (Fig. 1e) and the boundary between hepatocytes become invisible (Fig. 1f). While, patches of chronic lymphoid infiltration were observed with massive melanomacrophages and the blood sinusoids collapsed (Fig. 1f).

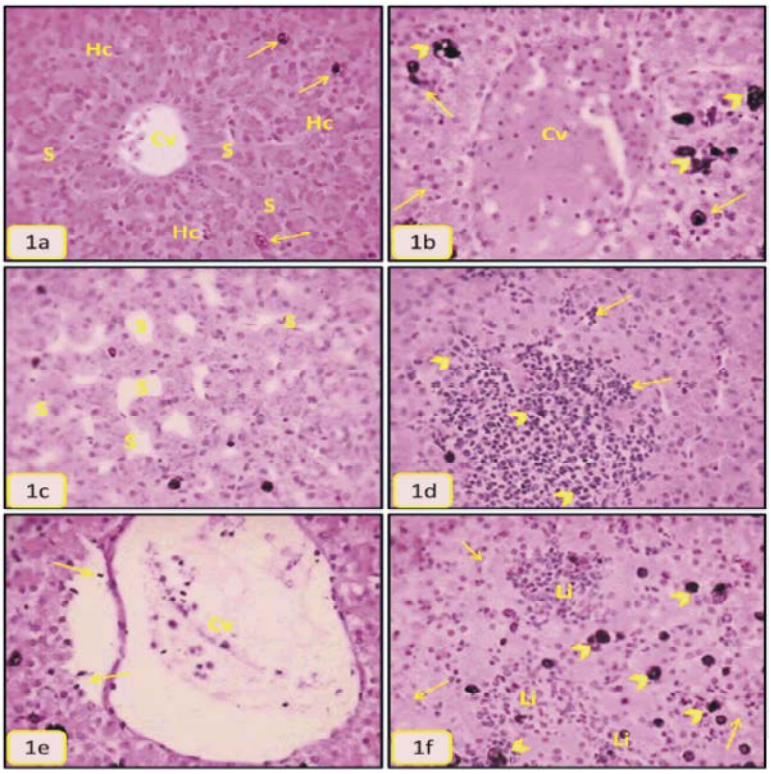

Fig. 1(a-f): Sections in toads liver tissues staining with (H&E): (a) liver tissue of the control group shows normal hepatocytes (Hc), few melanomacrophage (arrows), hepatic sinusoids (S) and central vein (Cv), (X400). (b-d) the tissues of a toad treated with 2.4 µg of 4 - Nonylphenol (acute dose) for four days. (b) shows the congestion of central vein (Cv) with necrosis of hepatocytes around it (arrows) and melanomacrophagespervasion (head arrows), (X400). (c) blood sinusoids dilation (S), (X400). (d) lymphocytes (arrows) and neutrophils aggregates (heads arrow), (X400). (e, f) the tissues of a toad treated with 0.48 µg of 4 - Nonylphenol (chronic dose) for a month. (e) separation of epithelial cells lining the central vein (arrows), (X400). (f) lymphcytes infiltration (Li), melanomacrophages (head arrows), blood sinusoids collapsed (arrows), (X400).

DISCUSSION

After exposure toad *Bufo arabicus* under study to different concentrations of a 4-Nonylphenol and then estimate the value of LC_{50} (lethal concentration half) found that the LC_{50} value is (the 96-hr LC_{50} = 4, 8 µ/l.) This Value indicates that the substance 4-Nonylphenol used in the experiment is more toxic to toad than other pollutants as the value listed with micron. In addition, the results of the current study revealed that the substance of the 4-nonylphenol more toxic for toad compared to values LC_{50} registered by the fish, with a toxic value of (LC_{50} = 0.32 ml/l) in the case of *Tilapia Oreochromisniloticuls* [25] and Satyanarayanan *et al*. [26] added that the value of the LC_{50} for catfish *Clariasgariepinus* exposed to material Nonylphenol was 3, 48 mg /l which indicates that the LC_{50} value vary between organisms and amphibians are most affected by pollutant and therefore used as evidence of biological water pollution [27].

The blood profile consider of the most important indicators that show the impact of pollutants on the functions of various organs of the organism [28, 29]. Current results indicate that the blood values of the control toads were located in the normal range as explained by Andersen *et al*. [30]. Ultscha *et al.* [31] pointed that the toads exposed to acute and chronic concentration of 4-nonylphenol revealed significant decreased in the number of red blood cells and content hemoglobin and the value of hematocrit and Mekkawy *et al.* [32] attributed this lack of blood constants to the decomposition of the red blood cells or deficiency in the production of red blood cells in the tissues that produce them. Considered to calculate the of blood indicators

(the average size of MCV, MCH and the MCHC have an important role to describe the state of anemia in most animals. In the present study was accompanied a lack of red blood cells and decrease hemoglobin content and in contras the hematocrit value and the blood cells size increased these finding has been proved by Coles [33] through histopathological studies which showed a decrease in tissue producing blood cells and the liver tissues necrosis with hemorrhage in the cases exposed to the concentration of acute and chronic 4-Nonylphenol. However, the changes that have occurred in the red blood cells volume may be a compensatory response to the hypoxic arising as a result of exposed to 4-Nonylphenol, as a means to increase the susceptibility of red blood cells to combine with oxygen [34]. It can be explained the increase in the number of white blood cells in current work as defense against bacterial infection in the affected organs, which is consistent with previous studies by Roche and Boge [35], or may be considered to catalyst white blood cells to increase the defenses cells [36].

That environmental pollution causes stress leads to cracking glycogen in the liver and thus raise the level of sugar in the blood [29, 32, 37]. In the current study, blood sugar levels increased significantly ($P<0.01$) in toads exposed to acute and chronic concentration of 4-Nonylphenol compared to the control group. Sayed *et al.* [38] and Mekkawy *et al.* [37], recorded the similar increase in the sugar level in the blood of many organisms exposed to diverse pollutants as ultraviolet radiation. Osman *et al.* [39] added that the chemical pollutants may alter the metabolic bath way of carbohydrates result in hyperglycaemia.

Enzymes such as plasma ALT, AST and ALP are a histologic enzymes may increase in the blood as a result of leakage from the cells in the infected tissue, therefore they used as evidence of some organs injury in the body [40, 41]. Accordingly, in the present study exposed Arab *Bufo arabicus* toad to acute and chronic concentration of 4-Nonylphenol led to a marked increase in amine transfer enzymes (AST, ALP) result of liver tissue degeneration. In addition to, the liver is rich in these enzymes and the injury led to the liberation of large quantities of them into the blood. The above results supports our histological findings in the liver of toads exposed to 4-Nonylphenol. Coincides with this study Satyanarayanan *et al.* [26] recorded that in the case of catfish that ALT and AST levels were increased with the concentration of 0.5 and 0.75 g/L of 4- Nonylphenol and then decreased with the concentration of 1 mg and proved that the increase in the levels of ALT, AST reflect liver injury. As previously mentioned by Bhattacharya *et al.* [42, 43], who pointed out that the 4- Nonylphenol may lead to indirect effect on the metabolic processes of these enzymes in the liver, kidney and bones tissues.

CONCLUSION

In light of the above, a significant correlations found between 4-Nonylphenol and liver tissue injure with functions efficiency and it was considered of interest to established research centers in different ecosystems in Saudi Arabia in order to(1) Examining the plants that fed on both sides of the waterway reaches for the detection of pollutants. (2) Separation of industrial waste and chemicals from sewage.

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