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Molecular Biomarker of heavy metal toxicity in *Lymnaea auricularia* (L.,1758) after acute exposure.

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***Abstract**: This study aims to figure out the heavy metals toxicity in Lymnaea auricularia after Fe acute exposure for different concentrations (1ppm, 5ppm, 10ppm, 15ppm), LT50 and LC50 were determined after 24,48,72,96 hr. respectively with detection of molecular biomarkers represented by DNA damage through DNA fragmentation test. The results showed that a highest LC50 was recorded in 0.6 ppm and highest LT50 equal to 71.05h in 1ppm, while the highest DNA damage was recoded as a highest lysis in 1 ppm after 96 hr. in this species with lysis level 2900.*

***Key words:** Fe toxicity, Lymnaea auricularia, DNA damage, acute exposure, LC50.*

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I. Introduction

Lymnaea auricularia (L.,1758) has health importance due to this species is considered an intermediate host for *Fasciola gignatica* (WHO,1995), also provide as an intermediate host for many danger trematodes species(Imani-Baranetal., 2011)The toxicity of iron is detect by absorption. The iron is absorbed in the (Fe^{2+}) state through intestinal mucouscells. Gastric and intestinal secretions can decrease ferric ions to the (absorbable) state**,** the significance of sentinel animal species for evaluating the potential impacts of heavy metals was previously studied by many researchers, toxicity study for Lymnea auricularia was done by Ali &Suliman (2012) when they used this species to evaluates the toxicity of copper sulfate, the results revealed that This that CuSO4.5H2O was highly effective in snail eradication and they recommended that the chronic effect of lower lethal dose of CuSO4.5H2O on the snails mortality and their effects on developmental stages should be investigated, and some local plant extract should also be tried for their potency on snail mortality.Coutellec&Lagadic (2006) have worked on Environmental factors that effect on stress and the Exposure to pollutants on Fitness-Related Traits of the Lymnaeastagnalis,and they found that effective initial family level heterogeneity for most measured characteristics, including physiological performances as detected through energetic biomarkers. Whatever the environmental circumstances, inbreeding depression was very low for progeny performances.

Metal promotes the toxicity and carcinogenicity with assistant of the role of the generation and role of reactive oxygen species (Valko etal.,2005), a new method was used to detect heavy metals toxicity by using Sensitivity of isolated eggs of pond snails which done by (Liu etal., 2013) and they found the sublethal effects in terms of a significant reduction in hatching rate could be found in the 25-µg/L treatment, and a significant decrease of heart rate was observed inboth treatments (5 and 25 µg/L).

II. Material and method

Tap water (Dechloride) was used in this experiments for acclimation of snail Lymnaea auricularia (L.,1758) with fixation of water quality parameters such as (Temperature:20 C°,pH:8.4, Dissolved oxygen:7.5 mg/l, T.D.S:0 mg/l, Salinity:0 ppt, E.C.:0 µs/cm), fish food adapted as nutrition for this species through acclimation period (Monzon&Kitikoon, 1991).Control species were used for comparison.

Four concentrations were used (1ppm, 5ppm, 10 ppm, 15 ppm) respectively and prepared from $FeCl^3$ stock solution provided by Merck Company (KGaA 64271 Darmstad , Germany).LC50 and LT50 were recoded after 24, 48, 72, 96 hr. for each concentration respectively.DNA damage was detect by gel electrophoresis after DNA extraction from Lymnaea auricularia (L.,1758) byDNA extraction Kit (CAT# A1120) used to identify DNA fragment and we followed the protocol clarified by Promega Corporation, Madison, WI , U.S.A, and after extraction ,DNA samples visualized by Electrophoresis in comparison with DNA ladder provided by Viogene company(Vioeasy^{TM}100 bp DNA ladder)

III. Result

During this experiments, mortality percentages were recoded after 24, 48, 72, 96 hr., after each concentrations (1ppm, 5 ppm, 10 ppm, 15 ppm), and the highest percentage was shown in 15 ppm after 96 hr. Table (1) elucidates the Mortality percentage for each concentrations.

Table 1: Mortality percentage for snail Lymnaea auricularia (L.,1758)which exposed to different concentration of Fe though exposure time.

Concentration ppm	Exposure time (hr.)	Mortality percentage %
1	24	**0.0**
	48	**16.6**
	72	**58.3**
	96	**79.1**
5	24	**0.0**
	48	**29.1**
	72	**66.6**
	96	**87.5**
10	24	**4.1**
	48	**41.6**
	72	**75**
	96	**95.8**
15	24	**8.3**
	48	**66.6**
	72	**95.8**
	96	**100**

Figure 1 showed that LC50 equal to 0.6ppm, LT50 in 1ppm was 71.05 hr. as appeared in Figure 2, 66.35 hr. was for 5 ppm after acute exposure as in figure 3,Figure 4 showed that LT50=50.7 hr. for10ppm of Fe after acute exposure, while for 15 ppm of Fe , LT50 was 38.49 hr.as clarified in Figure 5.

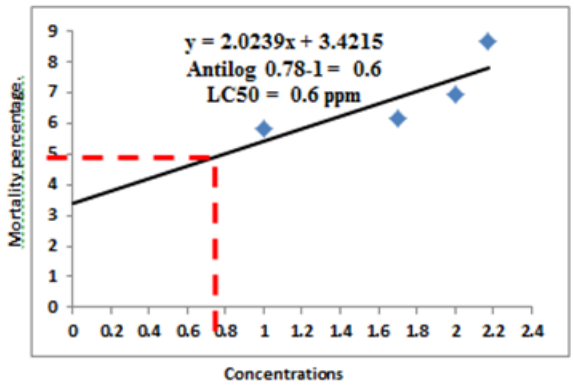

Figure 1:LC50 for Fe acute exposure experiments.

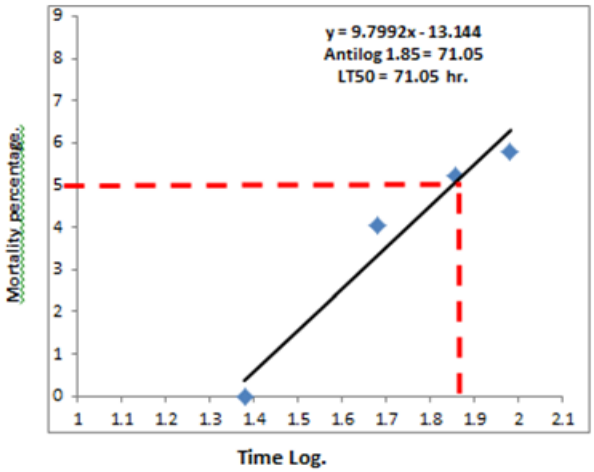

Figure 2:LT50 for Fe (1ppm) after acute exposure

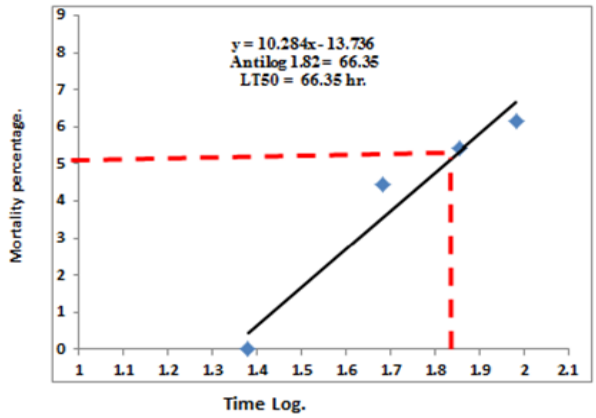

Figure 3:LT50 for Fe (5ppm) after acute exposure

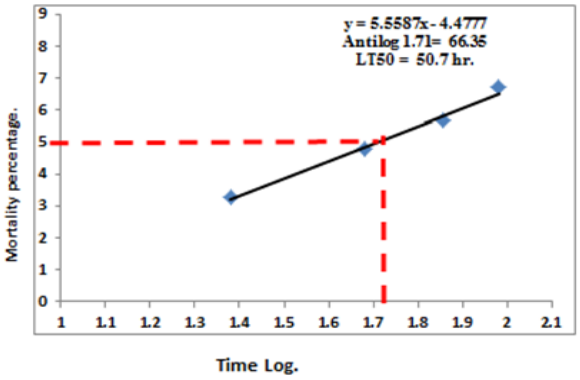

Figure 4:LT50 for Fe (10ppm) after acute exposure

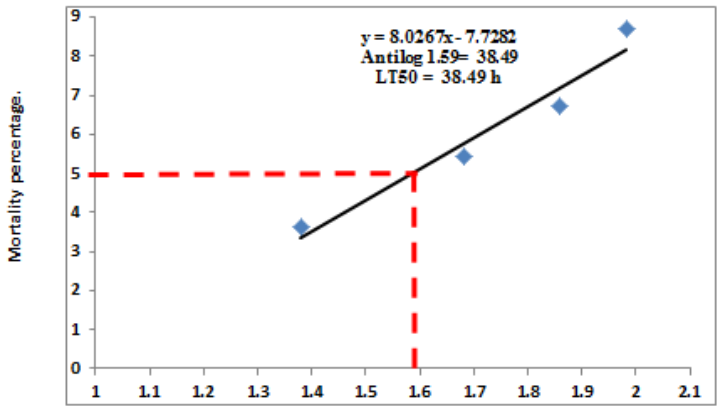

Figure 5:LT50 for Fe (15ppm) after acute exposure

Molecular biomarkers identified by DNA fragmentation test, the figure 6 showed that high damage appeared in all concentration after 96hr. especially for the 1 ppm which different a little bit than other concentrations.

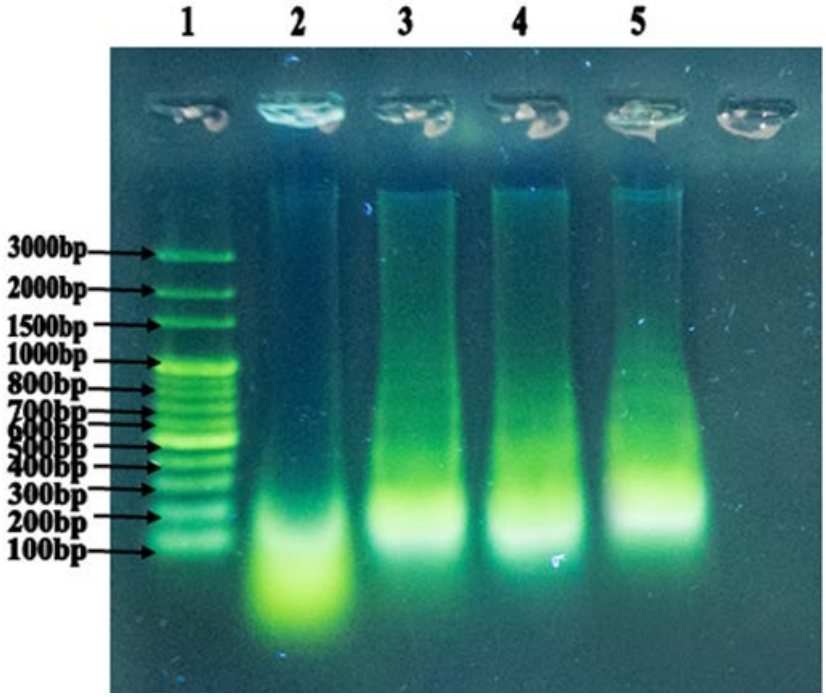

Figure 6: DNA damage in snail Lymnaea auricularia (L.,1758) induced by acute Fe exposure

1: DNA ladder (100bp), 2: 1ppm Fe, 3: 5 ppm Fe, 4: 10 ppmFe, 5: 15ppm Fe.

Table 2 showed that the highest lysis level was in 1ppm (2900) and so close to other concentrations, and this indication for toxicity effect pf iron on DNA of Snail species.

Table 2: Quantitative variations of DNA damage in snail Lymnaea auricularia (L.,1758) induced by acute Fe exposure

Lane number	Treatment	M.V(bp) Approx.	DNA lysis level
1	**DNA Ladder**	**100 bp**	**-**
2	**1ppm**	**3000-100 bp**	**2900**
3	**5ppm**	**3000-200bp**	**2800**
4	**10ppm**	**3000-200bp**	**2800**
5	**15ppm**	3000-200**bp**	**2800**

IV. Discussion

Metal toxicity renders crucial biological effects on an organism's survival, activity,growth, metabolism, or reproduction, heavy Metals can be affect the organism indirectly , the harmful effects onan organism's activity, growth, metabolism, and reproduction are found in sublethal effects (Wright and Welbourn, 2002).

Iron availability to organisms related with following: total concentrationof iron, chemical species, and the physicochemical properties of water (Xing& Liu,2011),Although we found DNA damage in this species but many literatures indicated that some species more resistant to lethal following exposure to sublethal concentrations which used in this study (Sheriiff& Delool,2001).In addition to that, heavy metals affect survival and physiological activities of experiment organisms including metabolic activities activities (Baby etal., 2010) and this lead to generation of ROS which lead to DNA damage which indicated in this experiment through DNA fragmentation test, many studies confirmed that heavy metals like Iron effect on DNA such as (Zhangetal.,2008) when indicated that a significant time-and dose-depended relationship between the heavy metal and DNA damage.

Two approaches effect on toxicity experiments, the first one is relationship between survival time of species and lethal factor level, and the second is exposure period (Sheriiff& Delool,2001).the mechanisms of DNA damage by iron can be summarized in two ways by produced of H2O2 through iron oxidation and OH production which react with DNA molecules and caused the fragmentation or an iron-independent pathway, Also iron have a vital role in production of Reactive oxygen species and activates peroxidative process(Gardietal.,2002)

V. Conclusion

We have confirmed that molecular biomarkers are a considerable indicator for detection of heavy metals toxicity and *Lymnaea auricularia* can be adapted as desirable biomarker species for heavy metals toxicity.

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