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Lead Nitrate induced morphological alteration of *Clarias batrachus* (Linn.1758) in Yamuna River of Brij Region

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ABSTRACT
We studied the effect of Lead nitrate, on Morphological parameters such as Body, Liver and Kidney weight of Clarias batrachus (Linn) under laboratory conditions. Yamuna River of Brij Region was selected for the collection of fish samples. The Fishes were collected from the different sites of study area and carried to the Toxicological laboratory of department. They were acclimatized and grouped for control and experimental set afterwards they were treated with stock solution of Lead Nitrate of 300, 400, 500, 600, 700 mg/L for 96 hours determination of LC50 values. It was determined as 505.17mg/L and Sub Lethal concentration of was 1/5th of test compound were 101.03mg/L. Experimental fishes were treated with different sub lethal concentration of Lead Nitrate for different time periods such as 24, 48, 72 and 96 Hours. The body weights of experimental set were found 253.33g, 230.00g, 211.66g and 183.33g while it is 280 g in control. Kidney and Liver were weighted after autopsy of both sets at different time intervals. Liver weight for experimental set was found 3.60g, 3.16g, 2.76g and 2.40g while it was 4.06g for control group similarly and Kidney weight 2.20g, 2.00g, 1.76g, 1.63 and 2.50g. The results revels that all the parameters were declining significant to very high significant with increase of exposure to lead nitrate
Keywords: *Lead Nitrate, Clarius batrachus, LC50, Morphological parameters, Yamuna River*

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INTRODUCTION

Pollution has become an inevitable part of the industries. Pollution takes its toll when the contaminant reaches to various levels of food chain and disturbs the balance of nature. The natural water bodies are incessantly being sloppy due to increase man-made activity and industrial operations of metal [8, 4]. According to Taylor *et al.,* [18] Lead and its compounds are most toxic metals and are included in the grey list of international conventions and well-known to cause harmful effects on aquatic animals [13]. The Holy River Yamuna is most polluted river in country and it is heavily polluted in National Capital territory Delhi and Brij Region, Mathura, Uttar Pradesh. There are several sources of lead nitrate effluents, such as leather tannery industry, anklet factories, battery disposing units, water Tap factories, industrial effluents, house hold and sewage or municipal discharge and pollute the river. Due to this pollution Aquatic biota severely affected. Fishes are most observant animal to heavy metal pollution [1]. They are well known creature in aquatic media hence mostly affected by pollution also they are used as bioindicators to monitor the water pollution, because they act in response with great sensitivity to changes in the quality aquatic fresh water bodies. [15].

The weight of body and visceral organs is important in forensic medicine and pathology, because the weight of internal organs is useful in determining whether the organ is normal or pathological. Changes in the weight of visceral body organs may be the only substantiation to give you an idea about that an organ is not normal [9, 12].

The present study deals with the outcome of lead nitrate on Body, Liver and Kidney of *Clarius batrachus,* (L.). In order to evaluate lead nitrate effects, we examined the LC 50 and body, liver and kidney weight exposed to different concentrations of test compound .

MATERIAL AND METHODS

Selection of study area

Four sampling sites (Ghats) of Yamuna River of Brij region, Mathura, were preferred from Downstream of Vrindaban to Farah town. The Sites were Keshi Ghat, Vishram Ghat, Gokul Barrage Ghat and Farah Ghat. These sites were chosen because these sites were heavily polluted by different kind of waste discharge Specially Lead Nitrate emission from silver anklet factories, commercial sectors, tannery industry and household discharge.

Rearing and Maintenance of fish

Live specimens were collected from the different sites and they were scrutinized for same size and length to justify the same age group and constant factors. Fish species were taxonomically identified in the laboratory according to Geldiay and Balık, [7]**.** All the specimen fishes were treated with 0.1 % potassium permanganate ($KMnO_4$) solution for 5 Minute to clarifying any viral, bacterial and fungal (if any) than they were reared in Glass aquaria (60cm X 25cmX 22cm) for about 21 days for acclimatization. The aquarium water was changed after two days or prior when it gives foul smell.

Test Compound

Lead (II)Nitrate $Pb(NO3)^2$, M.W.-331.20, Specific Gravity- 4.53 g/mL at 20°C was selected for present study.

The experiments were conducted into two phases-

Phase 1: Determination of LC50

Phase 2: Acute toxicity test

Determination of LC_{50}

For the ascertainment of LC50 in *Clarias batrachus (Linn)*, we divided them into 5 groups (A, B, C, D and E) in which each group consisted of 10 fishes. Lead Nitrate Standard stock solution was prepared by dissolving in water with the different strength 300,400,500,600,700mg/L. The mortality and survival number of fish was recorded for every concentration after 96 hours. The statistical analysis of data was done by log dose/probit regression line method [5]. For statistical analysis Regression line was drawn on the basis of two variables, log dose and empirical probit on the simple graph paper and used to determine the expected probit necessary for LC_{50} determination.

Organs Collection

The *Clarias batrachus* (Linn) were killed under light chloroform anesthesia. Body weight was weighted afterwards fishes were dissect out cautiously and the liver, kidney were taken out as per Gupta and Mullis 2010 the organs weighted separately. The weight was measured by digital weight measuring machine, manufacturer Manogyam 400-SF A.

Statistical calculations: In the present research, different statistical calculations were performed according as per Fischer and Yates [6].

RESULTS AND DISCUSSION

The toxic effect of lead nitrate on *Clarias batrachus* (Linn.) has been observed in the present study. From the collected data central tendency, mean ($\overline{X}$), standard deviation (SD) and standard error of mean (S.Em.) have been calculated and significance of difference has been tested by student't' test**.**

LC_{50} Value and Sub-Lethal Dose

In the current study the LC 50 Value was evaluated as 505.17mg/L for Lead Nitrate and The sub-lethal dose for test compound was $1/5^{th}$ of LC_{50} i.e. 101.03mg/L. The toxicity has been observed to be dose dependent.

Therefore it is concluded that it is concluded that all the morphological parameters viz. body weight , liver weight and kidney weight are dose dependent and their weight are significantly decline upon increase the concentration of Lead Nitrate. Maya [11] measured the toxicity of Roger to *Clarias batrachus* (Linn.) by studying its morphological and some other patho-physiological impact. Mubbunu *et.al* [12] also correlate of visceral Organ Weights with Body Weight in Normal Adult Zambians: A Case Study of Ndola Teaching Hospital. All findings are coinciding with Amjad [3] in which they explore the chronic exposure of lead leads to steady loss of the weight of body, Liver and Kidney. Rafique *et.al* [17] also finds out the decrease of visceral organs weight because nausea, vomiting and anorexia, which usually go together with exposure of heavy metals.

Body Weight: Body weight of the *Clarias batrachus* (Linn.) was took both at the start of the experiment and at the time of sacrifice. In lead-treated groups, there was steady weight loss in body weight with compared to control fish. The body weight ranged from 220-340 with an average of 280.0g. The body weight after intoxication of lead nitrate at after 24 hours was ranged from 210-300 with an average of

253.33g, while after 48 hours 190-225 with an average of 230.00g, while 72 hours ranged from 185-250 with an average of 211.66g, and after 96 ranged from 160-210 with an average of 183.33g.(Table-I, Fig-I). Nwochor, *et al.* [14] suggested that animals having a incessant exposure to heavy metals usually loses weight. However, overall deprivation of body weight with continuous introduction to lead might be explained on the basis of anorexia which is induced by ingestion of heavy metal [10]. These observations were also in conformity with the findings of Puvaneswari and Mohanambal [16].

Table-I: **Trends on Body weight in *Clarius batrachus* (L.) upon exposure of Lead Nitrate at different time intervals**

Body Weight (g)	Control	Exposure Time (In Hours)			
		24	48	72	96
RANGE	220-340	210-300	190-225	185-250	160-210
MEAN	280.0	253.33	230.00	211.66	183.33
±S.EM.	±34.64	±26.04**	±24.66**	±19.64***	±14.52***

S.Em.- Standard error of mean
*- Non-significant, **- Significant, ***- Highly significant ****- Very highly significant

Liver Weight: The liver is the key organ for metabolizing exogenous toxicants, but its role in Pb detoxification is not clear. The liver weight was ranged from 3.8-4.5 with an average of 4.06g for control group while the liver weight after treated with text compound at 24 Hours ranged from 3.2-4.1 with an average of 3.60g, after 48 hours it was ranged 2.8-3.6 with an average of 3.16g, after 72 hours it was ranged from 2.5-3.0 with an average of 2.76g, and after 96 hours ranged from 2.1-2.7 with an average of 2.40g, (Table-II,). The liver contains metelloroteins to which Pb may bind and this is the correlation that reduced liver weight is related to reduction with body weight [10].

Table-II : Trends on Liver weight in *Clarius batrachus* (L.) upon exposure of Lead Nitrate at different time intervals

Liver Weight (G)	Control	Exposure Time (In Hours)			
		24	48	72	96
RANGE	3.8-4.5	3.2-4.1	2.8-3.6	2.5-3.0	2.1-2.7
MEAN	4.06	3.60	3.16	2.76	2.40
±S.EM.	±0.21	±0.26*	±0.23**	±0.14***	±0.17***

S.Em.- Standard error of mean
*- Non-significant, **- Significant, ***- Highly significant,****- Very highly significant

Kidney Weight: The overall reduction in kidney weight is observed in our study. The kidney weight for control was ranged from 2.2-2.8 with an average of 2.50g, after treated with lead nitrate at 24 Hours ranged from 2.0-2.4 with an average of 2.20g, after 48 hours ranged 1.8-2.2 with an average of 2.00g, while after 72 hours ranged from 1.6-2.0 with an average of 1.76g, and after 96 hours it was ranged from 1.5-1.8 with an average of 1.63g. (Table-III). Afonne *et al* [1] observed reduce Kidney weight of mice after exposure of mercury hence present research is coinciding with him.

Table-III: Trends on Kidney weight in *Clarius batrachus* (L.) upon exposure of Lead Nitrate at different time intervals

Kidney Weight (g)	Control	Exposure Time			
		24	48	72	96
RANGE	2.2-2.8	2.0-2.4	1.8-2.2	1.6-2.0	1.5-1.8
MEAN	2.50	2.20	2.00	1.76	1.63
±S.EM.	±0.17	±0.11**	±0.11***	±0.12***	±0.08***

S.Em.- Standard error of mean
*- Non-significant, ** Significant, *** highly significant ****- Very highly significant

CONCLUSION

The body weight, liver weight and kidney weight showed decreasing trend on exposure to sub-lethal concentration of lead nitrate at different time intervals in *Clarias batrachus* (Linn). The decrease in body weight, liver weight and kidney weight with increase of exposure to lead nitrate is significant at 24 hours to very highly significant at 96hrs. This decline of body weight, liver weight and kidney weight is due the increase Lead Nitrate concentration in river water which become the cause of decrease in protein, lipid, and glycogen content also due to the binding of metellproteins with hepatocytes and nephrocytes which may damage the liver and kidney respectively.

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