# A STUDY ON DELETERIOUS EFFECT OF COPPER ON BLOOD OF CLARIASBATRACHUS

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## ABSTRACT

*This paper reports deleterious effect of copper on the blood of Clariasbatrachus by using $CuSO_4.5H_2O$ as the test chemical. The average median lethal concentration of 96hr-$LC_{50}$ of $CuSO_4.5H_2O$ was calculated to be 39.50 ( ≃ 40.00) mg/L. Two sub lethal doses i.e., 1/100$^{th}$ (=0.4 mg/L) and 1/200$^{th}$ (=0.2 mg/L) of $CuSO_4.5H_2O$ were selected for acute and chronic toxicity on blood of Clariasbatrachus.*

*Acute exposure of 0.4 mg/L of $CuSO_4.5H_2O$ for 24hrs, 48hrs and 96hrs duration showed a highly significant decrease from 2.33±0.10, 2.12±0.17 and 2.38±0.12×10$^6$/mm$^3$ respectively in comparison to control value 3.5875±0.15×10$^6$/mm$^3$of erythrocyte in Clariasbatrachus. In the cases of haemoglobin, acute exposure of $CuSO_4.5H_2O$ gave a significant decrease from 9.80±0.40, 9.67±0.22 and 10.21±0.28g/100ml respectively when compared to its corresponding control value 10.7625±0.47g/100ml. As far as, the variation in packed cell volume is concerned, the exposure of 0.4 mg/L of $CuSO_4.5H_2O$ for 24hrs, 48hrs and 96hrs duration leads to a highly significant decrease from 25.33±0.56%, 24.17±1.00% and 24.75±1.26% respectively in comparison to its control value of 27.943±0.9956% in Clariasbatrachus.*

*Similarly,chronic exposure of 0.2 mg/L of $CuSO_4.5H_2O$ for 15days, 30days and 45days duration showed a highly significant decrease from 2.13±0.04, 2.80±0.12 and 3.40±0.13×10$^6$/mm$^3$ respectively in comparison to control value 3.5875±0.15×10$^6$/mm$^3$ of erythrocyte in Clariasbatrachus. In the cases of haemoglobin, chronic exposure of $CuSO_4.5H_2O$ gave a highly significant decrease from 10.40±0.032, 9.50±0.20 and 10.10±0.08g/100ml respectively when compared to its control value 10.7625±0.47g/100ml. As far as, the variation in packed cell volume is concerned, the chronic exposure of 0.2mg/L of $CuSO_4.5H_2O$ for 15days, 30days and 45days duration leads to a highly significant decrease from 18.492±1.25%, 21.362±1.18%and 26.448±1.82% respectively in comparison to its control value of 27.943±0.9956% in Clariasbatrachus.*

*The observation of this study will help in establishment of suitable environmental condition of Clariasbatrachus.*

***Key Words: Clariasbatrachus, Acute Exposure, Chronic Exposure, Copper, Erythrocyte, Haemoglobin, Packed Cell Volume.***

## I. INTRODUCTION

Pollution of the aquatic environment with heavy metals has become a serious health concern in recent years (World Health organization, 1993; Ansari et al, 1994). These metals are introduced into aquatic ecosystem through various routes such as industrial effluents and wastes, agricultural pesticides, run off, domestic garbage dumps and mining activities (Merian, 1991). Increased discharge of heavy metals into natural aquatic ecosystems can expose aquatic organisms to unnaturally high levels of these metals (Van Dyk et al, 2007).

Among aquatic organisms, fish cannot escape from the detrimental effects of these pollutants, and are therefore generally considered to be the most relevant organisms for pollution monitoring in aquatic ecosystems (Van der Oost et al, 2003; Burger and Campbell, 2004).

The waste water from industries and also the sewage water from domestic sources containing heavy metals find their way into the nearby water bodies. Due to their persistence and accumulative nature, aquatic environment thus, absorb heavy metals from the surrounding contaminated water which ultimately affect their health. Among these animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa et al, 2004) and are therefore very susceptible to physical and chemical change, which may be reflect in their blood components (Wilson and Taylor, 1993).There are five potential routes for a pollutants to enter a fish. These routes are through the food, non-food particles, gills, oral consumption of water and the skin. The metal once absorbed is transported by the blood to either a storage point, such as bone or to the liver for transportation. If transported by the liver, it may be stored there, excreted in bile, or passed back into the blood for possible excretion by kidney or gills or stored in extra hepatic tissues such as fat. This is how heavy metal gets accumulated in different tissues of the fish via blood. Once heavy metals are accumulated by an aquatic organism, they can be transferred through the upper classes of the food chain and cause biomagnifications Cumine,1975;Mance,1987).

### 1.1 Materials and Methods

In the present investigation, a fresh water air-breathing teleost, *Clariasbatrachus* (Linn.) was taken as the experimental animal. *Clariasbatrachus* is a carnivorous air-breathing fish belonging to the family-Claridae and order-Siluriformes.

The live fishes were collected from local fisherman of Arrah and adjacent localities during 2012-2013. The fishes were brought to the laboratory in polythene bags containing the pond water.The fishes were disinfected by washing properly with dilute $KMNO_4$ and then transferred to many large aquaria (90×60×45 cm). The fishes were left for acclimatization to the laboratory conditions for a fortnight. During this period water of each aquarium was changed on alternate days. The fishes were fed with pieces of goat's liver and fish food available in local market.

Fishes of each aquarium were thoroughly examined and unhealthy and injured specimens were rejected. To keep the aquaria free from fungal growth antifungal chemicals were used. The temperature of water of each aquriam was maintained at room temperature throughout the period of investigation.

### 1.2 Determination of 96-hr$LC_{50}$ Dose

The toxicity tests of the heavy metals were conducted by employing the static bioassay method as designed by Doudoroft et al, (1951) and also recommended by APHA (1989) for the toxicity test experiments. As such, two sets of experiments, each were set up to determine the $LC_{50}$ doses for 24hrs, 48hrs, 72hrs and 96hrs of exposure for copper.

In each set of experiment, five different concentrations of copper were taken in separate aquarium. In each concentration, ten fishes form the acclimatized fish stock were kept. The first set of experiment was conducted for 24hrs, second for 48 hrs, third for 72hrs and fourth for 96hrs respectively. The experiments were monitored round the clock and numbers of fishes died during the experiments in each concentration of each set of experiment was recorded.

The above sets of experiments were repeated with fishes formed in the present project to determine the $LC_{50}$ dose of copper of the experimental fish. With the help of the records of dead fishes, $LC_{50}$doses for 24 hrs, 48hrs, and 72hrs and 96hrs exposures for copper were determined graphically by Probit Analysis (Finney, 1971). The behaviour of the fishes on exposure to various concentrations as well as to sub lethal dose of heavy metal was observed throughout the experiment.. The whole sets of experiment were repeated twice to get an average result and to minimize any error due to unavoidable reasons. Fishes were taken out for blood collection from the aquarium after the respective duration of treatment. Normal value of different blood parameters were collected before commencement of each toxicity test from the normal acclimatized fishes kept in separate aquaria and these fishes were called control groups. The values were considered as control values and were used for comparison with the experimental data's obtained from the treated fishes after conducting the toxicity test of each heavy metal. After determining the sub-lethal doses of copper selected for evaluation of their toxic effect on the haematology of *Cariasbatrachus*after treatment for different duration till 45 days.The median lethal concentration of 96hr -LC50 of $CuSo_4.5H_2o$ was calculated to 39.50 (≈40.00) mg/L. Two sub lethal doses i.e., $1/100^{th}$ (=0.4mg/L) and $1/200^{th}$(0.2mg/L) of 96hr-LC50 of $CuSo_4.5H_2o$ were selected for acute and chronic toxicity on blood of *Clariasbatrachus*.

## II. HAEMATOLOGICAL PARAMETERS

various haematological parameters namely total erythrocyte (RBC) count,haemoglobin (Hb )content, haematocrit value (packed cell volume or PCV) of *Clariasbatrachus* when expose to the sub lethal concentration of 0.4mg/L $CuSo_4.5H_2o$ for 24hrs, 48hrsand 96 hrs and 0.2mg/L of $CuSo_4.5H_2o$ for15 days, 30 days and 45 days exhibit distinct variations from the Control value of different haematological parameters. A detailed account of each parameter is given below:

## III. TOTAL ERYTHROCYTE (RBC) COUNTS

The normal value of total erythrocyte count in the controlled *Clariasbatrachus* was $3.5875\pm0.15\times10^6/mm^3$.The total erythrocyte count in fishes when exposed to the sub lethal concentration of copper showed following variations. The Acute exposure of 0.4 mg/L of $CuSo_4.5H_2o$ for 24hrs, 48hrs and 96hrs duration leads to ahighly significant decrease from $2.33\pm0.10$, $2.12\pm0.17$ and $2.38\pm0.12\times10^6/mm^3$ respectively in comparison to Control value $3.5875\pm0.15\times10^6/mm^3$ of Erythrocyte in *Clariasbatrachus*.

When duration of exposure was increased,.The Chronic exposure of 0.2 mg/L of $CuSo_4.5H_2o$ for 15days, 30days and 45days duration leads to a highly significant decrease from $2.13\pm0.04$, $2.80\pm0.12$ and $3.40\pm0.13\times10^6/mm^3$ respectively in comparison to Control value $3.5875\pm0.15\times10^6/mm^3$ of Erythrocyte in *Clariasbatrachus*.

## IV. HAEMOGLOBIN CONTENT

The haemoglobin content was analysed in *Clariasbatrachus* exposed to the sub lethal doses of copper for 24 hrs, 48hrs, 96hrs, 15 days, 30days and 45 days of exposure, a marked reduction was observed .The Acute exposure of 0.4mg/L of $CuSo_4.5H_2o$ for 24hrs, 48hrs and 96hrs duration leads to a significant decrease from $9.80\pm0.40$, $9.67\pm0.22$, $10.21\pm0.28$g/100ml respectively in comparison to Control value $10.7625\pm0.47$g/100ml of Haemoglobin in *Clariasbatrachus*

The Chronic exposure of 0.2mg/L of $CuSo_4.5H_2o$ for 15days, 30days and 45days duration leads to a highly significant decrease from 10.40±0.032, 9.50±0.20 and 10.10±0.08g/100ml respectively in comparison to Control value 10.7625±0.47g/100ml of Haemoglobin in *Clariasbatrachus.*

## V. PACKED CELL VOLUME (PCV) OR HAEMATOCRIT VALUE (HV):

The packed cell volume in the treated *Clariasbatrachus* showed a decreasing trend when exposed to different duration of copper. The Acute exposure of 0.4 mg/L of $CuSo_4.5H_2o$ for 24hrs, 48hrs and 96hrs duration leads to a highly significant decrease from 25.33±0.56%, 24.17±1.00% and 24.75±1.26% respectively in comparison to Control value of 27.943±0.9956% in Packed cell volume in *Clariasbatrachus*.

Whereas after exposure of *clariasbatrachus* to The Chronic exposure of 0.2mg/L of $CuSo_4.5H_2o$ for 15days, 30days and 45days duration leads to a highly significant decrease from 18.492±1.25%, 21.362±1.18% and 26.448±1.82% respectively in comparison to Control value 27.943±0.9956% of Packed cell volume in *Clariasbatrachus* .

## VI. RESULT

Acute Toxicity of Cupric Sulphate on Blood of *Clariasbatrachus*

| Parameter | Normal Range | Control | Period of Exposure | | |
|---|---|---|---|---|---|
| | | | 24 hrs | 48 hrs | 96 hrs |
| 1. Erythrocyte (x $10^6$/$mm^3$) | 0.58-7.06 | 3.5875±0.15 | 2.33±0.10 | 2.12±0.17 | 2.38±0.12 |
| 2. Haemoglobin (g/100 ml) | 6.40-21.20 | 10.7625±0.47 | 9.80±0.40 | 9.67±0.22 | 10.21±0.28 |
| 3. Packed cell Volume (%) | 11.80-60.86 | 27.943±0.9956 | 25.33±0.56 | 24.17±1.00 | 24.75±1.26 |

Chronic toxicity of Cupric Sulphate on Blood of *Clariasbatrachus*

| Parameter | Normal Range | Control | Period of Exposure | | |
|---|---|---|---|---|---|
| | | | 15 days | 30 days | 45 days |
| 1.Erythrocyte (x $10^6$/$mm^3$) | 0.58-7.06 | 3.5875±0.15 | 2.13±0.04 | 2.80±0.12 | 3.40 ±0.13 |
| 2. Haemoglobin (g/100 ml) | 6.40-21.20 | 10.7625±0.47 | 10.40±0.032 | 9.50±0.20 | 10.10±0.08 |
| 3. Packed cell Volume (%) | 11.80-60.86 | 27.943±0.9956 | 18.492±1.25 | 21.362±1.18 | 26.448±1.82 |

## V. SUMMARY AND CONCLUSION

The average median lethal concentration of 96 hr-LC50 of $CuSo_4.5H_2o$ calculated and its two sub lethal doses were selected for acute and chronic toxicity on blood of *Clariasbatrachus.*

The study showed that the effects of copper on blood of *Clariasbatrachus*. In erythrocyte it show significant decrease for both Acute and Chronic toxicity in comparison to their control value. In the cases of haemoglobin it show significant decrease for both Acute and Chronic toxicity in comparision to its control value. A significant decrease also studied in packed cell volume when compare to its control value.

Therefore it may be concluded that the changes in the haematological parameters indicate that, they can be used as indicators of copper stress in *Clariasbartrachus* on exposure to elevated copper level in water bodies. And contamination of water bodies by Heavy metals like copper is harmful for environment and their related organisms and it can create devastating situation in environment.

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