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EVALUATION OF ACUTE TOXICITY OF CATECHOL ALONG WITH HAEMATOLOGICAL, BIOCHEMICAL AND REPRODUCTIVE EFFECTS ON *CLARIAS BATRACHUS*

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ABSTRACT

The present study was conducted to evaluate the acute toxicity of catechol to Clarias batrachus along with its effects on haematological, biochemical and reproductive changes of fish. 96h median lethal concentration (LC50) of catechol along with its 95% confidence limits was 30.74 (30.02-31.56) mg/l. Haemoglobin (Hb), Total Red Blood Corpuscle Count (TRBCC) and Total White Blood Corpuscle Count (TWBCC) increased significantly ($p<0.05$) compared to the control (0.00 mg/l) at various days of exposure. A significant decrease ($p<0.05$) in total serum protein along with maturity index (MI) and significant increase ($p<0.05$) in total serum glucose was observed in treated fish for both the sublethal doses compared to control in case of catechol. The findings of the study may help forecast safe levels of catechol and portray the health status of fish in natural water bodies.

KEYWORDS : *Clarias batrachus*, Catechol, LC_{50}, Haematology, Biochemistry, Reproduction.

INTRODUCTION

Catechol or 1,2-Benzenediol is a phenolic compound which forms an essential constituent of various perennial trees like oak and willow along with fruits and vegetables comprising of apples, potatoes and olives[1,2]. It is used as an intermediate for synthesis of molecules for pharmaceutical and agrochemical industry, photography, rubber production, cosmetics, dyes, insecticides and oxidizing agent[3,4,5]. Besides, it has been found in wastewaters emanating from coal mining industry6. In Canada, it is one of the major byproduct of kraft process present in black liquor, an essential fraction of paper production[7]. Catechol reacts with DNA, proteins and membranes resulting in irreparable damage. It can lead to DNA adducts and strand breaks of chromosomes. It also readily interacts with proteins and enzymes resulting in their inactivation. The membrane-catechol interactions involve lipid peroxidation and uncoupling of oxidative phosphorylation. Some other chemical reactions of catechol involve complex formation with heavy metals, redox cycling and production of reactive oxygen species (ROS)8. Catechol has a low potential for bioconcentration in aquatic organisms but it readily adsorbs to suspended solids and sediment in water resulting in continuous exposure of this hazardous substance to various aquatic organisms9. Although there are some reports on the toxicity of catechol to both mammalian and fish models but there is scanty information on the specific effects of catechol on the haematological, biochemical and reproductive parameters of fish[10,11,12].

The present investigation is an attempt to determine96h median lethal concentrations (LC_{50}) of catechol to freshwater fish, *Clarias batrachus* along with its chronic effect on the various haematological, biochemical and reproductive parameters of fish. The study may help forecast some molecular mechanisms of catechol action as well as help to formulate plans for environmental monitoring strategies and ecosystem conservation measures.

MATERIALS AND METHODS

Adult walking catfish, *Clarias batrachus* of mean length 18.23 ± 0.64 cm and mean weight 113.6 ± 3.56 g were selected for the experiment. The fish was acclimatized to the test condition for a week before the start of the experiment. Analytical grade catechol, $C_6H_4(OH)$ (purity 99%, molecular weight 110.11 g/mol, Nice chemicals, Kochi, Kerala, India) was used as the test substance. Static replacement bioassays were used for both 96h acute toxicity tests and 45 days chronic toxicity tests[13]. The mean values of physicochemical properties of water used during the experiment were: temperature 26.4 ± 0.33 °C, pH 8.0 ± 0.52, free CO_2 4.7 ± 0.31 mg/l, DO 5.8 ± 0.28 mg/l, alkalinity 187.56 ± 3.74 mg/l as $CaCO_3$ and hardness 124.84 ± 3.89 mg/l as $CaCO_3$.

Acute toxicity tests for fish were conducted in 20 l glass aquaria holding 15 l of water in the laboratory. Four replicates were arranged for each concentration and each such replicate contained ten organisms. The fishes were not during the experimental period. After every 24h the number of dead fishes was counted and removed to avoid any organic decomposition and oxygen depletion within the test medium. The computer software R version 2.14.0[14] and probit analysis[15] was used for the determination of mortality rate at different concentrations and at different times of exposure. This was followed by the determination of 96h median lethal concentrations (LC_{50}) with 95% confidence limits of catechol to fish.

Chronic toxicity tests were conducted in 300 l cement vats in the outdoor for 45 days using two different sublethal concentrations (1.54 and 3.07 mg/l) of catechol and a control. The vats were arranged following standard procedures[16]. There were four replicates for each of the two sublethal doses and control. Each vat was stocked with ten adult *Clarias batrachus* for study on haematological, biochemical and reproduction of fish. In addition to natural food, the stocked fish were fed an equal mixture of fish meal, mustard oil cake and rice bran for a week @ 4% of their body weight. The blood samples along with the gonad weight of fish were analyzed at every 15 days interval for an entire exposure period of 45 days. The blood was drawn by puncturing the caudal vessels using a hypodermic syringe and collected in a vial containing EDTA as an anticoagulant. Haemoglobin (Hb), Total Red Blood Corpuscle Count (TRBCC), Total White Blood Corpuscle Count (TWBCC) and Mean Corpuscular Haemoglobin (MCH) were estimated17. Total Serum Protein (TSP) and Total Serum Glucose (TSG) was estimated using standard protocols[18,19]. Maturity Index (MI) were also determi ned[20,21].

All the chronic toxicity data were statistically analyzed using analysis of variance (ANOVA). Multiple mean comparisons were done by Duncan's Multiple Range Test (DMRT) using R version 2.14.0[14] and add-ins of Microsoft Excel 2007.

RESULT AND DISCUSSION

The 96h median lethal concentration (LC_{50}) with 95% confidence limits of catechol to fish is given in Table- 1. No organisms died in the control group during the experiment.

Table-1: Median lethal concentration (LC_{50}) in mg/l along with 95% confidence limits of catechol to *Clarias batrachus* at different hours of exposure (24, 48, 72, 96h)

Test organism	Concentration (mg/l)			
	24h	48h	72h	96h
Clarias batrachus (adult)	35.22 (34.90-35.92)	33.46 (32.85-34.35)	32.26 (31.16-33.18)	30.74 (30.02-31.56)

In the present study, 96h LC_{50} value of catechol to Clarias batrachus

(30.74 mg/l) was much higher than that observed in fathead minnow, *Pimephales promelas* (9.22 mg/l) and rainbow trout, *Oncorhynchus mykiss* (8.9 mg/l)[22,23]. The variation in the present values from the earlier studies was probably due to the variation in physicochemical parameters of the test medium[24]. The relationship between mortality rate and exposure times (24, 48, 72 and 96h) were found significant ($p<0.05$) at all the concentrations of catechol.

The mean values of various haematological, biochemical and reproductive parameters of fish exposed to sublethal doses of catechol are given in Table- 2.

Table- 2: Mean values (± SD) of haematological, biochemical and reproductive parameters of Clarias batrachus exposed to control (0.00 mg/l) and different sublethal concentrations (1.54, 3.07 mg/l) of catechol at different days of exposure (15d, 30d, 45d). The values are means of four replicates. Significant difference between any two means of a parameter is indicated by dissimilar superscript letters (DMRT at 5% level)

Parameters	Days								
	15			30			45		
	Dose (mg/l)								
	0.00	1.54	3.07	0.00	1.54	3.07	0.00	1.54	3.07
Hb (g/dl)	11.24an ± 0.07	11.31am ± 0.10	12.48bm ± 0.61	11.21an ± 0.22	11.28am ± 0.68	12.44bm ± 0.82	10.86am ± 0.25	10.91am ± 0.33	12.98bm ± 0.12
TRBCC (106/mm3)	2.98am ± 0.24	3.64bm ± 0.06	3.98bm ± 0.36	3.02am ± 0.31	3.78bn ± 0.06	4.14bm ± 0.34	3.02am ± 0.11	3.74bmn ± 0.08	4.24cm ± 0.11
TWBCC (102/mm3)	86.89am ± 3.53	112.44bm ± 4.12	138.58cm ± 4.46	87.12am ± 4.70	116.18bm ± 7.47	140.75cm ± 10.67	87.16am ± 3.45	117.56bm ± 5.59	144.81cm ± 4.29
MCH (pg)	3.79bm ± 0.30	3.11am ± 0.06	3.17am ± 0.41	3.74bm ± 0.35	2.98am ± 0.19	3.01am ± 0.08	3.60cm ± 0.09	2.92am ± 0.22	3.11bm ± 0.08
TSP (g/dl)	3.08am ± 0.09	3.01amn ± 0.07	2.98am ± 0.10	3.11bm ± 0.03	3.09bn ± 0.07	2.94am ± 0.13	3.18am ± 0.18	2.95am ± 0.04	2.92am ± 0.21
TSG (mg/dl)	26.85am ± 1.29	30.82bm ± 1.12	35.69cm ± 3.29	27.78am ± 1.62	31.27abm ± 3.43	37.78bm ± 6.41	29.91am ± 3.99	31.35am ± 3.47	40.22bm ± 3.92
MI	2.13cm ± 0.07	1.48bm ± 0.12	1.20am ± 0.10	2.11cm ± 0.08	1.45bm ± 0.20	1.16am ± 0.10	2.18bm ± 0.11	1.41am ± 0.19	1.21am ± 0.12

There was an increase in the Hb content in *Clarias batrachus* with the increase in concentration of catechol. The lowest and the highest values of Hb (10.86 and 12.98 g/dl) were observed for control and the higher chronic dose (3.07 mg/l) of catechol at the end of 45 days of exposure respectively. The Hb level at the higher sublethal dose (3.07 mg/l) increased significantly ($p<0.05$) compared to the control (0.00 mg/l) for all the days of exposure (15, 30 and 45 days). (Table- 2). The TRBCC of fish showed a proportionate increase with the two sublethal concentrations (1.54, 3.07 mg/l) of catechol compared to the control. TRBCC ranged from 2.98-4.24 million/mm^3. For all the concentrations of catechol tested including the control TRBCC peaked at the end of 45 days of exposure (4.24 g/dl). A significant increase ($p<0.05$) in TRBCC was observed among the different doses of catechol including the control only for 45 days of exposure (Table- 2). There was a marked rise in TWBCC of fish with the increasing concentration of catechol compared to control. TWBCC varied from 86.89-144.81 hundred/ mm^3. The maximum value was recorded for 3.07 mg/l at the end of 45 days and the minimum for control at the end of 15 days. A significant increase ($p<0.05$) in TWBCC were observed with the increase in concentration of catechol irrespective of the time of exposure (Table- 2). There was an overall decrease in MCH value of fish for both the sublethal concentrations (1.54, 3.07 mg/l) of catechol compared to control. MCH values ranged from 2.92-3.79 pg. During the different periods of exposure (15, 30 and 45 days), MCH at first showed a slight decline for the lower chronic dose (1.54 mg/l) and then increased slightly for the higher chronic dose (3.07 mg/l). A significant variation ($p<0.05$) in MCH value was observed among the different sublethal doses of catechol including the control only for 45 days of exposure (Table- 2). *Clarias batrachus treated* with catechol showed an increase in the Hb, TRBCC and TWBCC values and a decrease in MCH value compared to control. The increase in TRBCC was probably because of increased erythropoietin synthesis which in turn was due to high demand for O_2 or CO_2 transport. This resulted in an enhanced metabolic rate. Hypoxia stimulates oxygen transport which in turn increases Hb content25. Toxicant exposure results in stimulation of the immunological or defense system of the body which is reflected in the rise in TWBCC in the present investigation. Similar trend in TWBCC of fish exposed to different toxins was also reported by earlier workers[26].

A decline in TSP was observed in treated sets (1.54, 3.07 mg/l) compared to control (0.00 mg/l). The values varied from 2.92-3.18 g/dl. A significant variation ($p<0.05$) in TSP were observed between control and the higher sublethal dose only for 30 days of exposure (Table- 2). An increase in TSG was observed in treated sets (1.54, 3.07 mg/l) compared to control (0.00 mg/l). There was a significant increase ($p<0.05$) in TSG between control and the higher sublethal dose for all the days of exposure (15, 30 and 45 days) (Table- 2). Catechol probably suppresses serum protein production in fish by two different mechanisms which include inhibition of protein synthesis activity or disturbance in the incorporation of amino acids in the polypeptide chain[27]. Increased energy demand caused due to toxic stress also resulted in similar pattern of protein depletion in *Cyprinus carpio*[28]. The rapid breakdown of liver glycogen could have resulted in increased blood glucose levels in the present study. This is in agreement with the result obtained in *C. carpio* exposed to distillery effluent29. In addition, stress probably induces release of glucocorticoids and catecholamines in fish which causes the surge in blood glucose level[30].

Exposure to catechol results in reduction of MI of *C. batrachus.* A significant decrease ($p<0.05$) in MI was observed between the control and the higher sublethal dose (3.07 mg/l) of catechol (Table 2). The reduction of MI of *C. batrachus* subjected to chlorophenolic compounds may possibly reflect the reduction of the gonad mass. A similar trend in MI was observed in *Cyprinus carpio* when subjected to 4-tert-butylphenol and dichlorvos[31,32].

CONCLUSION

In summary, the present finding highlights the toxicity of catechol to fish during their acute and chronic exposure. These studies reveal the toxicant concentrations (viz. LC_{50}) that cause fish mortality even at short time exposure. The LC_{50} values of the present study may provide useful data to set up national and local water quality criteria (WQC) for catechol. The present haematological study shows that catechol poses a serious threat to the biological functions of fish during their chronic exposure. On the basis of haematological

studies, it would be possible to forecast no effect levels or safe levels of catechol discharged to the water bodies and portray the health status of fish in natural water bodies. Besides this, potential risk from the metabolites originating from catechol should be considered to get a more accurate picture in terms of toxicity.

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