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Assessment of Sub Lethal Toxicity of Hydrocortisone ($C_{21}H_{30}O_5$): Physiological and Haematological Biomarker Reactions on *Anabas testudineus*

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ABSTRACT

Application of wide range of pharmaceuticals is a serious threat to the environment causing a significant deterioration of aquatic faunal health as well as its survival status. The widespread detection of various types of drugs (antibiotics, non-steroidal anti-inflammatory drugs, beta-blockers, etc.) present in the aquatic ecosystem directly works on physiological parameters at different levels of aquatic organisms especially the bio-indicators and fishes. The experiment was carried out to evaluate the exposure of sub lethal concentration of hydrocortisone on oxygen consumption, gill movement, haematological and percentage of bone calcium of the freshwater fish (*Anabas testudineus*). When compared to control, the exposed fishes exhibit significant alterations in their physiological activity. The rate of oxygen consumption is indirectly proportional to dosage concentration as well as exposure time. Both the rate of gill movement and oxygen consumption were decreased with increasing concentration of hydrocortisone. Under exposure, the significant ($P < 0.05$) decline was recorded in both RBCs and Hb count when compared to control. The percentage of bone calcium level was significantly decreased by the toxicity of hydrocortisone. The current study stipulates that the exposure of sub lethal concentration of hydrocortisone on *A. testudineus* alters the physiological and haematological parameters. The results indicated that the steroid drug, hydrocortisone even at low concentrations causes deteriorative impacts on the health conditions of aquatic organism.

Key words: Hydrocortisone, oxygen consumption, gill movement, haematology, bone calcium, *Anabas testudineus*

INTRODUCTION

Pharmaceutical drugs are chemical substances designed to prevent, diagnose or treat various disorders and helps to promote health. The known biological effects of drugs on both humans and animals enhances its physical as well as mental well –being (More 2016, Karaman 2015). Now a day, the large scale manufacturing of different categories of drugs and its application are very prominent in the field of veterinary, agriculture and aquaculture for providing shelter against various diseases (Saravanan 2014). The classification of drugs are mainly focussed on three factors such as the chemical structure of a particular drug, the way it is used to treat a particular disease condition and its mechanism of action (Karaman 2015). Both the chemically synthesised drugs (termed as "small molecules") and living organism producing drugs (biologics) have advanced treatment capacity (Sarkis et al. 2020).

Therapeutic hydrocortisone is a synthetic or semi synthetic analogue of natural hydrocortisone hormone (Florey 1983). Though there are certain therapeutic advantages to hydrocortisone, it create several side effects at various respects such as headache, increased sweating, unusual hair growth on face or body, nausea, weight gain, skin dryness, rashes, Cushing syndrome, hyperglycaemia and seizures etc. The effects of hydrocortisone on various aspects of laboratory animals have been observed by a number of investigators (Sadasivudu 1977, Otomo et al. 1981, Edwards and Burnham 2001). Although there are some studies on the physiological aspects of cortisone (hydrocortisone) on rats, there

is lack of data for the effects of hydrocortisone on various physiological parameters of fresh water fishes.

The indiscriminate use of drugs can cause consequential impacts for the survival of organisms in the environment (Ambili et al. 2013, Boxall et al. 2004). Such emerging class of extensively used contaminants have potential effects on microbes, aquatic flora and fauna because they perform direct or indirect mode of action on various level (Fent et al. 2006 and Costanzo 2005). The ungovernable discharge of metabolites of pharmaceuticals from hospitals and other medical labs were accumulated in the surrounding water sources and that may lead to toxicity on biological aspects at a chronic level from target to non-target organisms (Calamari et al. 2003, Ferrari et al. 2004, Hermando 2006). The huge application of pharmaceuticals directly leads to the health status of the non-target species as they are biological indicators (Ahmad 2012, Chebbi 2010). Hence these chemicals are seriously affecting the vital organs as they are invading in the deteriorated water column (Al-Otaibia 2019).

The bioaccumulation of pharmaceuticals in various vital organs is primarily reflected in the rate of oxygen consumption, opercular frequency, haematological as well as percentage of bone calcium level. The rate of oxygen consumption is directly proportional to the metabolic rate of aquatic organisms and the changes in such sensitive process continuously leads to the behavioural as well as the physiological alterations also (Prasanna et al. 2020).The gills are considered as the remarkable area which supports the direct uptake of chemicals from the water column and hence it is an essential tool for the rapid assessment of both acute as well as the sub lethal toxicity with a real time manner (Afshan et al. 2014). In addition to that, the type of ventilatory responses exhibits by the gills indicates on what type of chemicals accumulated in that particular water column (Jerome et al. 1990). The haematological parameters are very sensitive to sub lethal concentration of pharmaceuticals by its rapid detection and hence any possible alteration in blood parameters are considered as one of the important biomarker of fish health (Haider and Rauf 2014, Alimba 2019). Generally the bone density and calcium level are significantly depends on various types of drugs and hence the regular consumption of drugs have contribute the gradual bone resorption (Vestergaard 2008).

Anabas testudineus (climbing perch) was selected as a model organism for this study because such native fish commonly inhabiting in rice fields and it is considered as one of the important food fish also (Tam et al. 2015). Acute and chronic toxicities of several pesticides have been investigated in climbing perch (Binoy et al. 2004, Choudhury et al. 1993, Cong and Linh 2010, Jilna and John 2011), but information regarding the effects of drugs is scarce. No information is available on the impacts of hydrocortisone on physiological as well as haematological aspects in climbing perch. The objective of this work is to study the effect of sub lethal toxicity of hydrocortisone on opercular frequency, oxygen consumption; haematology and determination of bone calcium level of *A. testudineus* using certain biomarkers.

MATERIALS AND METHODS

Collection and maintenance of test animal

Live and healthy specimens of climbing perch (*Anabas testudineus*) obtained from Keezhillum fish farm, were used in the experiment. Average weight of the fish was 20±5 gm. Fish were put into experimental aquarium for acclimatization at least 14 days before the beginning of the experiment. During this period, water in the aquarium was aerated permanently and water temperature was regulated at 23±1°C by using thermostatic heaters. The fish were fed with a locally manufactured fish food to satiety, twice daily during the period of acclimation. The aquarium water conditions like pH, dissolved oxygen and hardness were analyzed weekly as per the standard recommendations.

Sub lethal toxicity tests

The acclimatised fishes were exposed to two different sub lethal concentrations (5 and 10 ml/L) of hydrocortisone for different time intervals (100, 300 and 500 hr). The fishes were divided two groups as control and treatment group. A control group is simultaneously run along with the treated groups of hydrocortisone. Each experiment was repeated six times and more than 100 fishes were used for the

study. The dosed fishes with different sub lethal concentrations were maintained for different time intervals for the stress analysis. The fishes were regularly fed with fish food and the feeding was stopped before two days prior to the experiment.

Oxygen consumption

The dosed fishes were blotted dry and weighed to the nearest mg to calculate the metabolic rate. The dissolved oxygen was estimated adopting Modified Winkler Method (Strickland and Parsons 1972). The oxygen consumed by the fish is expressed as ml/L/min/gm. The breathing rate was checked periodically from first minute to sixty minutes of exposure by recording the opercular frequency with visual counting and the average values were calculated.

Haematological studies

The fishes treated with different concentrations of hydrocortisone were dissected out and blood is collected directly from the heart with an insulin syringe for the analysis of haematological parameters. The collected blood is transferred to heparinised vials and analysed. The red blood cells were counted with the aid of standard haemocytometer after diluting the blood with Dace's solution (Blaxhall and Daisley 1973). The estimation of haemoglobin was done by the cyano-methaemoglobin method (Larsen and Snieszko 1961)

Determination of bone calcium

The two sets of exposed fishes along with control were used for the analysis of percentage of bone calcium. The determination of bone calcium was performed by the standard chemical analysis contains two treatment methods. The treated bone powder were volumetrically analysed (Huang 1989) by atomic absorption spectroscopy (Eugien et al. 2014).

Statistical analysis

The experimental data obtained were analyzed using Excel and SPSS. Students''t' test was applied to calculate the significance level of oxygen uptake between the control and hydrocortisone exposed fishes. The P value is less than 0.05, denotes that the values were statistically different. Six samples for each category were obligated for analysis. The standard deviation and correlations were calculated by using SPSS. The graphs were plotted using MS Excel.

RESULTS AND DISCUSSION

Opercular frequency and oxygen consumption

The ventilation rate was directly proportional to the dosage concentration at different time interval of exposure. The documented rate of opercular movement was found to be elevated in dosed fishes with 5 ml/L and 10 ml/L hydrocortisone, when it was

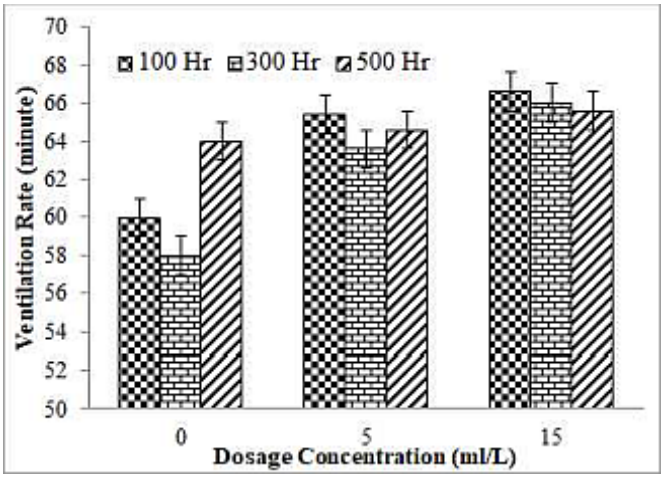

Figure 1. Ventilation rate in control and different dosage of hydrocortisone after 100, 300 and 500 hrs of exposure on *A. testudineus*

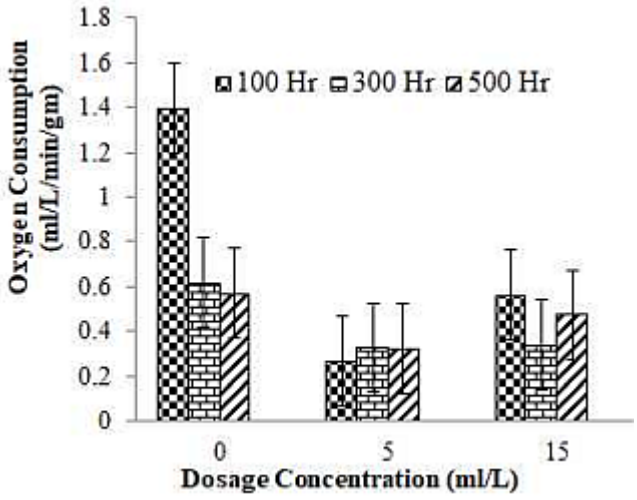

Figure 2. Oxygen consumption in control and different dosage of hydrocortisone after 100, 300 and 500 hrs of exposure on *A. testudineus*

compared to the opercular rate of control fishes at various hours (100, 300 and 500) of exposure. The gill movement of control fishes at 100 hr of exposure showed 60 per minute, whereas the dosed fishes (5 and 10 ml/L) exhibited 65.4 and 66.6 per minute after the same period of exposure. The control fishes with 300 hr of exposure revealed a gill movement of about 58 per minute while both the treated groups with 5 and 10 ml/L dosage have gill movement of 63.6 and 66 per minute, respectively. The manifested rate of opercular movement of control fishes at 500 hr of exposure indicated a rate of 64 per minute, but in both the dosed groups it was 64.6 and 65.6 per minutes (5 and 10 ml/L). The dosed groups in both 300 and 500 hrs of exposure exhibits a decreased rate of gill movements than the fishes treated with hydrocortisone at 100 hr of exposure. Due to the gradual accumulation of hydrocortisone, the dosed fishes with increased time exposure showed decreased rate of gill movement (Fig.1). The results of oxygen consumption of *Anabas testudineus* exposed to different concentration of hydrocortisone were summarized in Figure 2. The oxygen uptake of 100 hr exposure in control was 1.3964 ml/L/min/gm, in 5 ml/L it was 65.4 ml/L/min/gm and in 10 ml/L it was 66.6 ml/L/min/gm. The control group with 300 hr of exposure showed an oxygen consumption of 0.6158 ml/L/min/gm whereas both the treated fishes have an oxygen consumption of 63.6 ml/L/min/gm in 5ml/L and 66 ml/L/min/gm in 10ml/L, respectively. The consumption of oxygen with 500 hr of exposure indicated the values of 0.5712 ml/L/min/gm in control, 64.6 ml/L/min/gm in 5ml/L and 65.6 ml/L/min/gm in 10 ml/L. In control group, the average rate of oxygen uptake gradually decreased when the time of exposure reaches from 100 to 500 hours. Similarly the oxygen consumption of dosed fishes exhibits declined rate when compared to control as the exposure time goes on (Fig. 2).

By comprehensively examining the gill movement that supported the fish to take more aeration after the exposure of toxicant (drug), were able to explain the detrimental effects of sub lethal toxicity. Since the gills considered the main respiratory organ in fishes, the alterations in gaseous exchange is directly or indirectly depends on toxicants accumulated in the water column. The chemical properties of the changed water medium will be reflected in animal's ventilator activity (Mushigeri 2003). Fishes exhibited drastic changes on ventilatory movement in the initial phase and it comes down towards the sub lethal level of toxicant because of its direct accumulation (Patil et al. 2003). The fishes revealed a decreased respiratory rate in sub lethal medium as they were accommodate the new chemical environment (Murthy 2013). The significant increases of opercular movements in the initial stage by the exposure of toxicants gradually decreased because the fishes tried to compensate the increased physiological activities under stressful conditions (Shivakumar and David 2004). Such respiratory distress because of the disfigurement of oxidative metabolism has been clearly reported in *Tilapia mossambica* under the stress of cypermethrin (Marigoudar 2009). Similar report of fluctuated ventilatory movements was observed in blue gill sunfish under the chronic exposure of heavy metal compounds and chlorinated hydrocarbon substances (Diamond et al. 1990). Results obtained from the present study clearly proved that the exposure of hydrocortisone at sub lethal concentration provides the decreased rate of oxygen consumption in increased exposure time when compared to the control groups. The main reason behind the reduced oxygen consumption of the fish is due to the weakened oxidative metabolism by the occurrence of respiratory distress (Logaswamy and Remia 2009, Marigoudar 2009).

Different studies were reported among various toxicants on different fishes have the same observation (Vani et al. 2020, Lokhande 2017, Vidya et al. 2016). The exposure time of toxicant was indirectly proportional to the rate of oxygen consumption in *Pseudetroplus maculates*, under the sub lethal toxicity of bisphenol due to the mucous accumulation on the gill as well as the shrinkage of the respiratory epithelium (Asifa et al. 2018). The observation of a significant alteration in respiratory rate and reduction of oxygen consumption in both lethal and sub lethal concentration of sodium cyanide is due to the impaired oxygen metabolism was reported in *Labeo rohita* (David et al. 2015). The correlation exists in between the rate of toxicant uptake and the oxygen consumption of fishes without considering the fish size and species were also reported earlier (Yang et al. 2000). The altered

respiratory activity of *L. rohita* with increased exposure time of chromium may be due to the depression in the metabolic rate (Vutukuru 2005).

The general well-being of the fish is determined by the consumption of total oxygen as an indicator in *Cirrhinus mrigala* and the increasing respiratory activity mainly influenced by the sub lethal exposure also (Veni and Veeraiah 2014). Similar findings were reported in different fishes under exposure to different toxicants (Prashanth et al. 2003, Magare and Patil 2000, Susan et al. 2010, Neelima 2016, Kumari and Rao 2018).

Haemoglobin and RBC count

The changes in haematological parameters of *A. testudineus* exposed to control and different doses of hydrocortisone have been summarized in Figure 3. The Hb count of control group was 10 Gm % at 100 hr, 300hr and 500 hr of exposure. The values of Hb at 5ml/L exposure of hydrocortisone was 9.1, 9 and 8.2 Gm % under 100, 300 and 500 hr of exposure, respectively. The dosed group with 10 ml/L of hydrocortisone showed an Hb values of 9, 8.9 and 8 Gm % after 100, 300 and 500 hr of exposure. The Hb concentration in both 5 ml/L and 10 ml/L hydrocortisone decreased with increasing time of exposure. The embodied data from the table revealed that the gradual decreased value of Hb is depends on both increasing concentration of drug as well as the period of exposure in each dosage group. Hence there was a significant negative correlation is existing between dosage and haemoglobin count ($P<0.05$). The changes in red blood corpuscles of *A. testudineus* exposed to control and different doses of hydrocortisone have been encapsulated and indicated that the fishes were exposed to different concentration exhibits decreased values as compared to control fishes (Fig. 4). The RBC count of fishes with controlled condition after 100 hr of exposure was 11.79 million/cmm, while dosed fishes with same condition exhibited the values of 11.05 and 10.51 million/cmm at 5 ml/L and 10ml/L hydrocortisone, respectively. The decreased RBC values of 10.69 million/cmm (5ml/L) and 10.22 million/cmm (10ml/L) at 300 hr of exposure was recorded in the fishes, while the control treatment fishes with the same hour of exposure maintained the value 11.9 million/cmm. The treated fishes of both the concentrations (5 and 10 ml/L) let out the minimum values of 5.46 and 4.78 million/cmm after 500 hr of exposure, whereas the control group showed a value of 11.79 million/cmm. A drastic change of RBC value was noted after 500 hour of exposure in both 5 ml/L (5.46 million/cmm) and 10 ml/L (4.78 million/cmm) of hydrocortisone. The value of red blood corpuscles decreased with increasing concentration of hydrocortisone shows a significant difference exist in between both dosage concentration as well as the value of red blood corpuscles ($P<0.05$).

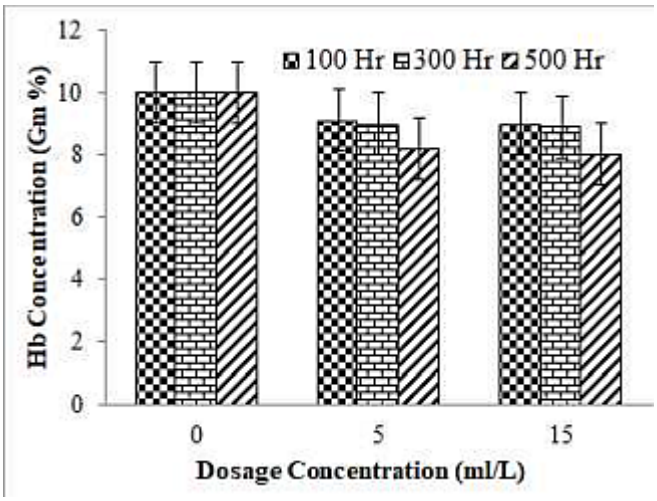

Figure 3. Hb count in control and different dosage of hydrocortisone after 100, 300 and 500 hrs of exposure on *A. testudineus*

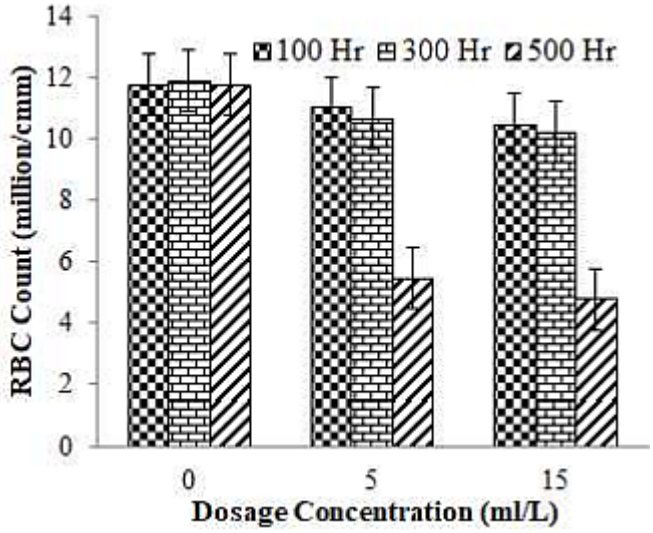

Figure 4. RBC count in control and different dosage of hydrocortisone after 100, 300 and 500 hrs of exposure on *A. testudineus*

The haematological responses of both RBC and Hb concentration were decreased under the sub lethal toxicity of hydrocortisone throughout the study period. Similarly, a depletion of total RBC count in the blood of common carp (*Cyprinus carpio)* when exposed to clofibric acid and diclofenac (Saravanan 2011). The decreased counts of erythrocytes were observed in Indian major carp under the toxic effect of ibuprofen when compared to their respective control groups (Saravanan et al. 2012). The alterations in certain haematological parameters between exposed and control group of various fishes were analysed and the decreased levels of both RBC and Hb were also observed (Umamaheswari et al. 2019, Nwani et al. 2016).A significant reduction ($P < 0.05$) of RBC and Hb concentration among the dosed fishes of *L. rohita* fingerlings were observed under the exposure of N-acetyl-p-aminophenol toxicity, the content of paracetamol drug (Renuka et al. 2018). The *C. carpio* exhibited the decreased erythrocytes counts were reported under the exposure of carbamazepine (Rezaei *et al*. 2020). Nwani et al. (2014) discussed the effects of different concentration of chloramphenicol on blood parameters and the significant reduction in the value of Hb occurred in *Clarias gariepinus.*

The haematological study in the rain bow trout revealed declined values of both Hb and RBC parameters causes anaemia under the exposure of verapamil (Li et al. 2011). The trending changes of haematological parameters were observed with the exposure of *L. rohita* in oxytetracycline showed a significant reduction of RBC value as they were anaemic (Ambili et al. 2013).

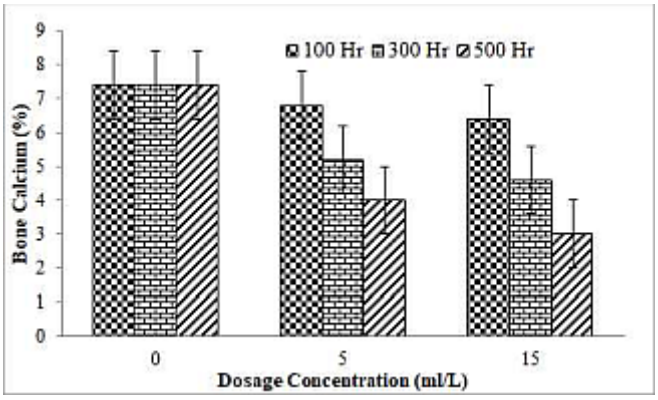

Figure 5. The value of bone calcium in control and different dosage of hydrocortisone after 100, 300 and 500 hrs of exposure on *A. testudineus*

Determination of bone calcium

The percentage of bone calcium obtained from *A. testudineus* treated with different dosage of hydrocortisone at different duration of exposure was detailed in Figure 5. The average bone calcium level at control group was 7.40 % in 100, 300 and 500 hours of exposure. Comparatively, the dosed groups recorded lower level of calcium percentage in both 5 and 10 ml/L when the time of exposure goes on. In 5ml/L of hydrocortisone concentration, the average value of calcium is in between 6.80 to 4 % as the time of exposure increases from 100 to 500 hr. Besides this, the dosed fishes with 10ml/L hydrocortisone shows higher value (6.40 %) at 100 hour of exposure and it was gradually decreased to 4.60 and 3.00 % at 300 and 500 hour of exposure, respectively (Fig. 5).

Regarding the sub lethal toxicity of pharmaceutical drugs, there were no reports prior to the present study, among the toxicity of fresh water fishes in the aspects of bone calcium determination. In this study, the treated groups of fishes with hydrocortisone exhibited a decreased level of bone calcium than the control groups as the time of exposure increased and that tendency continuous gradually till the end of the experiment. The earlier studies had reported a decreased level of serum/ plasma calcium in treated fishes by the exposure of different toxicants (Srivastav et al. 2009, Srivastav et al. 2010, Alam et al. 2014) and also the impaired bone metabolism is due to the low-dose or occasionally administered glucocorticoid therapy (Gröber and Kisters 2012).

CONCLUSION

The findings behind the exposure of hydrocortisone on major physiological parameters indicate that the steroid drug, hydrocortisone is toxic to *Anabas testudineus*, and even at low doses it can alter the ventillatory, haematology and percentage of bone calcium profiles of this fish. The values of oxygen consumption, gill movement, haematological parameters and bone calcium level exhibit a decreased physiological activity when the exposure time and the concentration of drug increased.

Therefore, it is concluded that hydrocortisone at sub lethal concentration can cause considerable worsening of fish health status as well as its survival.

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REFERENCES

Afshan, S., Ali, S., Ameen, U.S., Farid, M., Bharwana, S.A., Hannan, F. and Ahmad, R. 2014. Effect of different heavy metal pollution on fish. Research Journal of Chemical and Environmental Sciences, 2(2), 74-79.

Ahmad, Z. 2012. Toxicity bioassay and effects of sub-lethal exposure of malathion on biochemical composition and haematological parameters of *Clarias gariepinus*. African Journal of Biotechnology, 11(34), 8578-8585.

Alam, A., Tabinda, A.B., Hassan, M.U. and Yasar, A. 2014. Comparative toxicity of acetamiprid and azadirachtaindica leave extract on biochemical components of blood of *Labeo rohita*. Pakistan Journal of Zoology, 46(6), 1515-1520.

Alimba, C.G, Adekoya, K.O, Soyinka, O.O. 2019. Exposure to effluent from pharmaceutical industry induced cytogenotoxicity, hematological and histopathological alterations in *Clarias gariepinus* (Burchell, 1822). EXCLI Journal, 18, 63-78.

Al-Otaibia, A.M., Al-Balawia, H.F.A., Ahmada, Z. and Suliman, E.M. 2019. Toxicity bioassay and sub-lethal effects of diazinon on blood profile and histology of liver, gills and kidney of catfish, *Clarias gariepinus*. Brazilian Journal of Biology, 79 (2), 326-336.

Ambili, T.R., Saravanan, M., Ramesh, M., Abhijith, D.B. and Poopal, R.K. 2013. Toxicological effects of the antibiotic oxytetracycline to an Indian major carp *Labeo rohita*. Archives of Environmental Contamination and Toxicology, 64(3), 494–503.

Asifa, K.P. and Chitra, K.C. 2018. Sublethal toxic effects of bisphenol A on oxygen consumption, haematological and histological parameters in the cichlid fish, Pseudetroplus maculatus (Bloch, 1795). International Journal of Zoological Investigations, 4(1), 21-30.

Binoy, V.V, Nishitha, J. and Thomas, K.J. 2004. Influence of dicofol on the behaviour of the climbing perch, *Anabas testudineus*. Indian Journal of Fish, 51(3), 345–351.

Blaxhall, P.C. and Daisley, K.W. 1973. Routine haematological methods for use with fish blood. Journal of Fish Biology, 6(5), 771-781.

Boxall, A.B.A., Fogg, L.A., Blackwell, P.A., Kay, P., Pemberton, E.J and Croxford, A. 2004. Veterinary medicines in the environment. Reviews of Environmental Contamination and Toxicology, 180, 1-91.

Calamari, D., Zuccato, E., Castiglioni, S., Bagnati, R. and Fanelli, R.2003. Strategic survey of therapeutic drugs in the rivers Po and Lambro in northern Italy. Environmental Science and Technology, 37(70), 1241–1248.

Chebbi, S.G. and David, M. 2010. Respiratory responses and behavioural anomalies of the carp *Cyprinus carpio* under quinalphos intoxication in sub lethal doses. Science Asia, 36(1), 12-17.

Choudhury, C., Ray, A.K. and Bhattacharya, S. 1993. Non-lethal concentrations of pesticide impair ovarian function in the freshwater perch, *Anabas testudineus*. Environmental Biology of Fishes, 36, 319–324.

Cong, N.V. and Linh, N.T. 2010. The possibility of using muscle cholinesterase in climbing perch (*Anabas testudineus*) as biomarker of effects of diazan 60EC application on rice fields at Binh Thuy district, Can Tho city [in Vietnamese]. Cantho University Press, Cantho City; 165–172.

Costanzo, S.D., Murby, J. and Bates, J. 2005. Ecosystem response to antibiotics entering the aquatic environment. Marine Pollution Bulletin, 51(1-4), 218–223.

David, M., Sangeetha, J. and Harish, E.R. 2015. Sodium cyanide induced alteration in the whole animal oxygen consumption and behavioural pattern of freshwater fish *Labeo rohita*. Journal of Environmental Biology, 36(2), 405-408.

Diamond, J.M., Parson, M.J. and Gruber, D. 1990. The observation of different ventilation responses indicated the presence and type of toxicants in the environment and bluegill sunfish. Environmental Toxicology and Chemistry, 9, 3-11.

Edwards, H. and Burnham, W. 2001. The impact of corticosteroids on the developing animal. Pediatr Research, 50, 433–440.

Eugien, X.L., Pravinkumar, M., Logesh, A.R., Viswanathan, C. and Raffi, S.M. 2014. Estimation of calcium and phosphorus in bones of three low value fishes Sardinella fimbriata, Sardinella albella and Sardinella gibbosa from Muttom, Southeast Coast of India. International Journal of Current Research, 3, 43-46.

Fent, K., Weston, A. and Daniel, C. 2006. Ecotoxicology of human pharmaceuticals. Aquatic Toxicology, 76(20), 122–159.

Ferrari, B., Mons, R., Vollat, B., Fraysse, B., Paxeus, N. and Giudice, L.R. 2004. Environmental risk assessment of six human pharmaceuticals: are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment? Environmental Toxicology and Chemistry, 23(5), 1344–1354.

Florey, K. (Eds), 1983. Analytical Profiles of Drug Substances. Academic Press, 12, 277-324.

Gröber, U. and Kisters, K. 2012. Influence of drugs on vitamin

D and calcium metabolism. Dermato-Endocrinology, 4(2), 158-166

Haider, M.J. and Rauf, A. 2014. Sub-lethal effects of diazinon on hematological indices and blood biochemical parameters in Indian carp, *Cirrhinus mrigala* (Hamilton). Brazilian Archives of Biology and Technology, 57(6), 947-953.

Hernando, M.D., Mezcua, M., Alba, F.A.R. and Barcelo, D. 2006. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta, 69(2), 334–342.

Jerome, M., Diamond, M., Arolyn, J., David, P.G. and Ruber, D.G. 1990. Rapid detection of sub lethal toxicity using fish ventilatory behavior. Environmental Toxicology and Chemistry, 9(1), 3-11.

Jilna, A.N. and John, T.K. 2011. Effect of sub lethal concentration of methyl parathion on the behavioural activities of the climbing perch (*Anabas tesudineus* : bloch). Advances in Applied Science Research, 2, 167–172.

Karaman, R. 2015. Commonly used drugs - uses, side effects, bioavailability & approaches to improve it. Nova Science Pub Inc; UK ed. Edition, 292: ISBN: 978-1-63463-828-9.

Kumari, T.K. and Rao, G.G. 2018. Effect of phenthoate 50% ec on oxygen consumption of freshwater fish *Catla catla*. International Journal of Science and Research, 7(11), 1515-1521.

Larsen, H.N. and Snieszko, S.F. 1961. Comparison of various method of determintion of haemoglobin in trout blood. Fish Culture, 23(1), 8-7.

Li, Z.H, Velisek, J., Zlabek, V., Grabic, R., Machova, J., Kolarova, J., Li, P.andRandak, T. 2011. Chronic toxicity of verapamil on juvenile rainbow trout (*Oncorhynchus mykiss*): effects on morphological indices, hematological parameters and antioxidant responses. Journal of Hazardous Materials, 185(2-3), 870-880.

Logaswamy, S. and Remia, K.M. 2009. Impact of cypermethrin and ekalux on respiratory and some biochemical activities of a freshwater fish, *Tilapia mossambica*. Journal of Current Biotica, 3(1), 65-73.

Lokhande, M.V. 2017. Oxygen consumption and behaviour surveillance in the freshwater fish *Rasbora daniconius* exposed to dimethoate. International Journal of Fisheries and Aquatic Studies, 5(2), 712-716.

Magare, S.R. and Patil, H.T. 2000. Effect of pesticides on oxygen consumption, red blood cell count and metabolites of fish, *Puntius ticto*. Journal of Ecology and Environment, 18, 891–894.

Marigoudar, S.R., Ahmed, R.N. and David, M. 2009. Cypermethrin induced respiratory and behavioural responses of the freshwater teleost, *Labeo rohita* (Hamilton). Veterinarskiarhiv, 79(6), 583-590.

More, B. 2016. Overview of medicine- its importance and impact. DJ International Journal of Medical Research, 1(1), 1-8.

Murthy, K.S., Kiran, B.R and Venkateshwarlu, M. 2013. A review on toxicity of pesticides in Fish. International Journal of Science and Research, 1(1), 15–36.

Mushigeri, S.B. 2003. Effect of fenvalerate on the metabolism of in Indian major carp, Cirrhinusmrigala. Ph.D. Thesis. Karnatak University, Dharwad, Karnataka India.

Neelima, P., Rao, N.G., Rao, G.S. and Rao, J.C.S. 2016. A study on oxygen consumption in a freshwater fish *Cyprinus carpio* exposed to lethal and sub lethal concentrations of cypermethrin (25%Ec). International Journal of Current Microbiology and Applied Sciences, 5(4), 338-346.

Nwani, C.D., Mkpadobi, B.N., Onyishi, G., Echi, P.C., Chukwuka, C.O., Oluah, S.N. and Ivoke, N. 2014. Changes in behavior and hematological parameters of freshwater african catfish *Clarias gariepinus* (Burchell 1822) following sublethal exposure to chloramphenicol. Drug and Chemical Toxicology, 37(1), 107-113.

Nwani, C.D., Odo, G.E., Nwadinigwe, A.O., Onyeke, C.C., Atama, C.I., Ngwu, G., Oluah, S.N., Ukonze, J.A and Ezeibe, B.C.A. 2016. Short-term effects of albendazole on the oxidative stress markers and hematological parameters in tissues of african catfish *Clarias gariepinus*. Journal of Aquatic Animal Health, 28, 222-228.

Otomo, S., Higuchi, S., Nakaike, S., Takeshita, K., Tanaka, M., Gotoh, Y., Osada, Y., Tsuchida, K., Inoue, K., Kyogoku, K., Tarumoto, Y., Sasajima, M. and Ohzeki, M. 1981. Pharmacological study on hydrocortisone 17-butyrate 21-propionate. Folia Pharmacologica Japonica, 78 (6), 647–658.

Patil, J.A., Patil, A. and Govindwar, S.P. 2003. Biochemical effects of various pesticides on sprayers of grape gardens. Indian Journal of Clinical Biochemistry,18, 16–22.

Prasanna, C., Anithasmruthi, C. and Rathnamma, V.V. 2020. A study on oxygen consumption in freshwater fish *Labeo rohita* exposed to lethal and sub lethal concentrations of ethion 50% Ec. Indian Journal of Forensic Medicine & Toxicology, 14(2), 122-127.

Prashanth, M.S., David, M. and Kuri, R.C. 2003. Effect of cypermethrin on toxicity and oxygen consumption in the freshwater fish, Cirrhinus mrigala. Journal of Ecotoxicology and Environmental Monitoring. 13(4), 271–277.

Renuka, S., Poopal, R., Ramesh, M. and Bindu, F.C. 2018. Responses of *Labeo rohita* fingerlings to n-acetyl-p-aminophenol toxicity. Ecotoxicology and Environmental Safety, 157, 73-80.

Rezaei, M., Moradi, A.M, Mortazavi, P. and Jamili, S. 2020. Effects of chronic exposure to carbamazepine on hematological parameters in *Cyprinus carpio*. Iranian Journal of Fisheries Sciences, 19(1), 443-456.

Sadasivudu, B., Rao, I.T, Radha, C. and Murthy, K. 1977. Metabolic effects of hydrocortisone in mouse brain. Neurochemical Research, 2(5), 521-32.

Saravanan, M. 2014. Pharmaceutical residues in India: impact on aquatic environment. Current Science, 107(10), 1634-1635.

Saravanan, M., Devi, K.U., Malarvizhi, A. and Ramesh, M. 2012. Effects of ibuprofen on hematological, biochemical and enzymological parameters of blood in an indian major carp, *Cirrhinus mirgala*. Environmental toxicology and pharmacology, 34(1), 14-22.

Saravanan, M., Karthika, S., Malarvizhi, A. and Ramesh, M.

2011. Ecotoxicological impacts of clofibric acid and diclofenac in common carp (*Cyprinus carpio*) fingerlings: hematological, biochemical, ionoregulatory and enzymological responses. Journal of Hazardous Materials, 195, 188-194.

Sarkis, M., Bernardi, A., Shah, N. and Papathanasiou, M.M. 2021. Emerging challenges and opportunities in pharmaceutical manufacturing and distribution. Processes, 9(3), 2-16.

Shivakumar, R. and David, M. 2004. Toxicity of endosulfan to the freshwater fish, *Cyprinus carpio*. Indian Journal of Ecology, 31, 27–29.

Srivastav, A.K., Srivastava, S.K, Mishra, D. and Suzuki, N. 2009. Effects of deltamethrin on serum calcium and corpuscles of stannius of freshwater catfish, *Heteropneustes fossilis*. Toxicological & Environmental Chemistry, 91(4), 761-772.

Srivastav, A.K., Srivastava, S.K., Mishra, D. and Srivastav, S. 2010. Deltamethrin-induced alterations in serum calcium and prolactin cells of a freshwater teleost, *Heteropneustes fossilis*. Toxicological and Environmental Chemistry, 92(10), 1857-1864.

Strickland, I.D. and Parsons, T.R. 1972. Practical handbook of seawater analysis. Bull. 167(second edition), 203.

Susan, T.A., Sobha, K. and Tilak, K.S. 2010. A study on acute toxicity, oxygen consumption, and behavioural changes in the three major carps, *Labeo rohita* (ham), *Catla catla* (ham) and *Cirrhinus mrigala* (ham) exposed to fenvalerate. Bioresearch Bulletin, 1, 35–43.

Tam, N.T, Berg, H., Tuyen, P.T.B.T. and Cong, N.V. 2015. Effect of chlorpyrifos ethyl on acetylcholinesterase activity in climbing perch (*Anabas testudineus*, Bloch, 1972). Archives of Environmental Contamination and Toxicology, 69(4), 1-132.

Umamaheswari, S., Renuka, S.S, Ramesh, M.andPoopal, R. 2019. Chronic amoxicillin exposure affects *Labeo rohita*: assessment of hematological, ionic compounds, biochemical, and enzymological activities. Heliyon, 5(4), 1-25.

Vani, G., Veeraiah, K., Kumar, M.V. and Parveen, S.K. 2020. Effect of cartap hydrochloride (50% sp) on oxygen consumption of fresh water fish, *Cirrhinus mrigala* (Hamilton). Pollution Research, 39(2), 368-372.

Veni, S.S.M. and Veeraiah, K. 2014. Effect of cypermethrin (10%ec) on oxygen consumption and histopathology of freshwater fish *Cirrhinus mrigala* (Hamilton). IOSR Journal of Environmental Science, Toxicology and Food Technology, 8, 12–20.

Vestergaard, P. 2008. Adverse effects of drugs on bone and calcium metabolism/physiology. Clinical Reviews in Bone and Mineral Metabolism, 6(1), 1-16.

Vidya, P.V., Asifa, K.P. and Chitra, K.C. 2016. Effect of silica-nanoparticles (SiO^2-nps) on oxygen consumption in freshwater fish, *Oreochromis mossambicus* (PETERS, 1852). Journal of Global Biosciences, 5(1), 3611-3614.

Vutukuru, S.S. 2005. Acute effects of hexavalent chromium on survival, oxygen consumption, haematological parameters and some biochemical profiles of the indian major carp, *Labeo rohita*. International Journal of Environmental Research and Public Health, 2(3), 456–462.

Yang, R., Brauner, C., Thurston, V., Neuman, J. and Randall, D.J. 2000. Relationship between toxicant transfer kinetic processes and fish oxygen consumption. Aquatic Toxicology, 48(2-3), 95-108.

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