Acta Biologica Turcica, 34(1), 1-8, 2021 Received 25 Jun. 2020; Accepted 21 Oct. 2020

ACTA BIOLOGICA TURCICA

E-ISSN: 2458-7893, http://www.actabiologicaturcica.com

Research article

Effect of oral contraceptive pills (mala-d) on melanophores of some fresh water fishes

Somashekar Danayakapura SHIVRUDRAPPA[1], Majagi SHASHIKANTH[2,*]

[1]*Department of PG Studies in Zoology, I.D.S.G. Government College, Chikkamagaluru-577102*

[2]*Department of studies in Zoology, Vijaynagar Sri Krishnadevaraya University, Ballari Karnataka-India-583105*

**Corresponding author: smajgi@rediffmail.com*

Abstract: Aquatic toxicologists acknowledge that effects of drugs on chromatophores are effectively used as an indicator of pollution. The movement of melanophores with in the skin cells is responsible for pigmentation, which involves the nervous system as well as the endocrine system. An attempt is made to evaluate the impact of the oral contraceptive pill (OCP:MALA-D) on melanophores of some fresh water fishes Viz., Common carp (*Cyprinus carpio*),*Catla catla* and *Labeo rohita* .The present results revels that the OCP (Mala-D) have action on the melanophores and disturbing in their structure. In all the above mentioned fish species three types of melanophores were observed (Punctate,Stellate Reticulostellate).The number and shape of each type of melanophores significantly altered with the increase of time of exposure. In *L. rohita* the initiation of the disappearance of melanophores observed in 96hrs of exposure. In the present investigation the results indicates that OCP (MALA-D) affects the external surface area and also endocrine system so melanophores indicate stress condition.

Keywords: Melanophores, Oral contraceptive pill, Fresh water fishes, *Cyprinus carpio*), *Catla catla*, *Labeo rohita*

Citation: Somashekar, D.S., & Majagi, S.H. (2021). Effect of oral contraceptive pills (mala-d) on melanophores of some fresh water fishes. *Acta Biologica Turcica,* 34(1), 1-8.

Introduction

The rapid growth of pharmaceutical industry in India has posed an elevated risk of environmental contamination because of their extensive disposal from medical centers and discharge of domestic water. The physiologically active nature of pharmaceuticals, however, raised concerns about their potential impacts on non-target species when they were inadvertently discharged into the ecosystem (Fent et al., 2006; Ankley et al., 2007). Research has shown that the environmentally relevant concentrations of pharmaceutical drugs cause toxicological health impacts on various aquatic organisms in general and fishes in particular. More importantly, studies on the potentially adverse ecological impacts of pharmaceutical drugs and their residues on the physiology of aquatic organisms are scarce in India (Saravanan, et al., 2011). For instance, Larsson et al. (2007) have reported elevated concentrations of pharmaceutical drugs such as ciprofloxacin, losartan, cetirizine, metoprolol, enrofloxacin, citalopram, norfloxacin, lomefloxacin, enoxacin, ofloxacin and ranitidin (range between 90 and 31,000 g/l) in the effluent of a sewage treatment plant in Patancheru Enviro Tech Ltd (PETL), Patancheru, Hyderabad, India.

Saravanan, et al. (2013) and co-workers reported toxicological effects of clofibric acid (lipid regulating prodrug), diclofenac (a non-steroidal anti-inflammatory drug) and ibuprofen (analgesic, antipyretic and anti-inflammatory) in an Indian major carp, *Cirrhinusmrigala*and *C. carpio*. Ambili et al. (2013),

observed significant alterations on hematological and enzymological responses of an Indian major carp *Labeorohita*exposed to oxytetracycline (antibiotic). Thus, detailed and targeted investigations are required to study the sources, pathways and fate of the pharmaceutical drugs (Rehman, et al., 2015).

Most oral contraceptives use 17-α Ethinylestradiol (EE2) as a synthetic replacement for estrogen. When women use these contraceptives they typically absorb 80% of the EE2 dose and waste 20% (Zhang et al., 2008). Wastewater treatment plants receive this EE2-containing effluent and remove some organic wastes, but not all of the EE2 (Fent et al., 2006). This treated effluent then re-enters the water table via movement from the plant into surface waters. EE2 concentrations in these released effluents have been linked to significant negative histological and reproductive effects on aquatic organisms, including fish (Fent et al., 2006).

Melanophores are the pigment containing and light reflecting cells; they are groups of cells, found in bacteria and a wide range of animals including amphibians, fishes, reptiles, crustaceans and cephalopods. Mammals and birds in contrast have a class of cells called melanocytes for coloration. Melanocytes are cells located in the bottom layer, the basal lamina, of the skin's epidermis and in the middle layer of the eye, are controlled either by nerves alone or by a combination of nervous and endocrine system. (Bagnara & Hadley, 1973; Fuji & Oshima, 1994; Fuji, 2000). Chromatophores are largely responsible for generating skin and eye color in cold blooded animals and are generated in the neural crest during embryonic development.

The movement of these chromatophores within the cells is responsible for the pigmentation or color change in the skin. Melanophores transport their pigment in response to extracellular cause, neurotransmitters in the case of fish and hormonal stimuli in the case of frogs. In both cases, melanosomes dispersion is induced by elevation of intracellular cAMP levels, while aggregation is triggered by depression of cAMP (Reiter, 1985). The pharmacological studies also suggested the presence of cholinergic receptors which mediate dispersion of pigment in the melanophores of fishes. The effect of acetylcholine on the fish melanophores has been a subject of debate for long time (Parker, 1948; Scott, 1965; Miyashita and Fujii, 1973).

The available literature survey shows that less work has been made in order to know the impact of oral contraceptive pills (MALA-D) on the fresh water fishes. Keeping in this view the study was undertaken on the fresh water edible fishes of Common carp (*Cyprinuscarpio*), Catla (*Catlacatla*), and Rohu (*Labeorohita)*, fishes which are an important food source in many parts of the country including Karnataka.

Materials and Methods

The fresh water fishes (*Labeorohita*), Catla (*Catlacatla*)and Common carp (*Cyprinuscarpio*) were collected from Yagachi reservoir about 25 km from Chikkamagaluru, Karnataka.

The 10 scales of each species of the above fishes mentioned were removed from dorso-lateral region below the head, tail and lateral sides of the fish using forceps. Then the scales were immediately transferred into the plastic containers containing lethal concentration of oral contraceptive pills (MALA-D), (4.7grms/ltr) at different hours (24hrs, 48hrs, 72hrs, and 96hrs). Along with the exposure, the control scales were maintained in the containers containing physiological saline.

After each particular time exposure, the scales were plucked and immediately perfused with the physiological saline which had the following composition in mm (NaCl: 12.8, KCl: 2.7, CaCl 2:1.8, Glucose, 5.6 and NaOH with pH value 7.4). Then the scales were placed on a clean glass slide for the observation of the drug effect on the response of certain groups of melanophores. Studied with camera fitted stereo binocular microscope under low power (10x), the different kind of chromatophores were counted in 10 scales of each species at lateral region and the photographs were taken.

Results

The fresh water fishes (*Catlacatla, Cyprinuscarpio*, and *Labeorohita*) scales exposed to lethal concentration (4.7g/lt) of oral contraceptive pills (MALA-D) showed that the decrease in the number of melanophores from control to 96 hrs duration of time of exposure and alteration of shape of melanophores were also observed in the lateral region of scales.

In the scales of *Catlacatla,* the three types of melanophores were observed punctate, stellate and reticulo stellate, their number gradually decreasing with the increase in the time of exposure from control to 96 hrs (Plate-01). The punctate type of melanophores was seen in 48 hrs and 72 hrs of exposure 31.2±0.196 and 19.2±0.196 respectively. The stellate melanophores were

recorded in 24 and 48 hrs of exposure and their observation was 42.1±0.240 and 34.3±0.26 respectively. The reticulo stellate melanophores were observed in 24, 48 and 96 hrs of exposure and their readings were 31.2±0.296, 24.9 ±1.085, 18±0.099 respectively. The reticulo stellate type of melanophores was observed in controlled scales about 31.2±0.296 (Table 1 and Fig. 1).

Table 1. Changes on the melanophores of *Catlacatla, Cyprinuscarpio*and*Labeorohita* exposed to lethal concentration (48hr) of OCP's (4.7g/lt).

Species	Exposure period day/hours	Punctate	Stellate	Reticulo stellate
Catlacatla	Control			31.2±0.296
	24hrs	-	42.1±0.240	24.9 ±1.085
	48hrs	31.2±0.196	34.3±0.26	-
	72hrs	19.2±0.196	-	-
	96hrs	-	-	18±0.099
Cyprinuscarpio	Control	-	-	53.22±2.587
	24hrs	24.2±0.970	51.2±2.587	-
	48hrs	-	35.4±0.787	38.5±0.580
	72hrs	13.6±3.88	21.5±0.580	-
	96hrs	-	-	14.9±0.242
Labeorohita	Control			42.4 ±0.04
	24hrs	-	-	29.4 ±0.084
	48hrs	33.6±3.88	31.2±0.196	18.8±0.196
	72hrs	22±3.88	20.9±0.242	-
	96hrs	-	-	11.9±0.242

Each value is the mean of 10 individuals, ± = Standard error

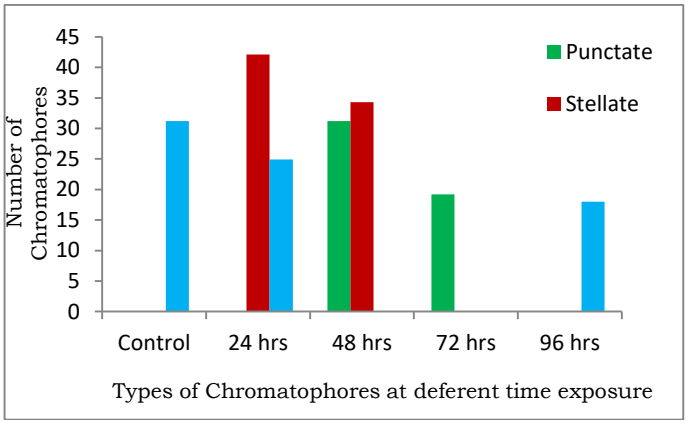


Figure 1. Changes on the melanophores of fish *Catlacatla*

In the scales of *Cyprinuscarpio*also three types of melanophores were observed (punctate, stellate and reticulo stellate); their number gradually decreased with the increase in the time of exposure from control to 96 hrs (Plate-02). In 24 hrs of exposure the punctate and stellate melanophores were recorded 24.2±0.970 and 51.2±2.587 in number respectively. The stellate and reticulo stellate melanophores were recorded about 35.4±0.787 and 38.5±0.580 respectively in the 48 hrs of exposure. In 72 hours of exposure punctate and stellate melanophores were recorded as 13.6±3.88 & 21.5±0.580 in number respectively. Only reticulo stellate melanophores were observed in both control (53.22±2.587) and 96 hrs (14.9±0.242). (Table 1, Fig. 2).

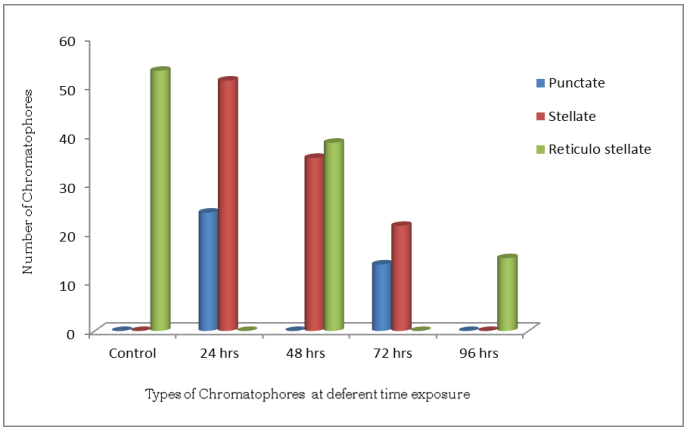


Figure 2. Changes on the melanophores of fish *Cyprinuscarpio*

In the scales of *Labeorohita*the reticulo stellate melanophores were observed in duration of exposure except in 72 hrs, the number is gradually decreased from control to 96 hrs (42.4 ±0.04 in control, 29.4 ±0.084 in 24 hrs, 18.8±0.196 in 48 hrs and 11.9±0.242 in 96 hrs). In 96 hrs of exposure the initiation of disappearance of reticulo stellate melanophores was observed. Punctate and stellate melanophores were recorded only in 48hrs (33.6±3.88

and 31.2±0.196) and 72 hrs (22±3.88 and 20.9±0.242) of exposure respectively, their number also decreased with the increase in the time of exposure (Plate 3, Table 1, Fig. 3).

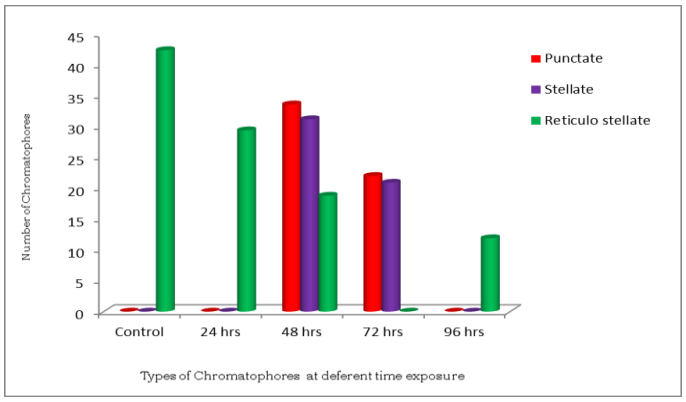


Figure 3. Changes on the melanophores of fish *Labeorohita*

Discussion

The pharmacological studies suggested the presence of cholinergic receptors that mediate dispersion of pigment in the melanophores of fishes. The effect of acetylcholine on the fish melanophores has been a subject of debate for long time (Parker, 1948; Scott, 1965; Miyashita & Fujii, 1973).

The movement of the melanophores within the skin cells is responsible for the pigmentation of the skin; such type of the movement of the melanophores involves nervous system as well as endocrine system. Chemical messengers or neurotransmitters are released from the neuron terminals to control this dispersion and aggregation phenomenon (Sinha, et al., 1999). Considerable variations among fishes in the normal mechanism of control (Parker, 1943). Watanable et al. (1965) and Praveen et al. (1993) noticed the effect of alkali ions in several fish species. These ions directly affect the sympathetic post ganglionic nerve terminals release the neurotransmitters, which induce the aggregation of the melanophores within the cells. Several studies on the effect of hormones, chemicals and drugs on chromatophore (Yamada et al., 1984).

The present investigation revealed that the lethal concentration (4.7g/lt) of oral contraceptive pills (MALA-D), has dispersiv action on the melanophores and disturbing in their structure at early hours of exposure, while uniform arrangement of melanophores were noticed in the controlled scales. The study involved the observation of the scales of three important fresh water species *Catlacatla*, *Labeorohita* and *Cyprinuscarpio*andin all fishes three types of melanophores were observed (punctate, stellate and reticulo stellate). The number of each type of melanophores decreased with the increase of the time of exposure from control to 96 hrs. In *Labeorohita*the initiation of disappearance of melanophores was observed in the 96 hrs of exposure. Therefore, the lethal concentration (4.7g/lt) of the contraceptives affect the external surface area and also the nervous systems, so melanophores indicate the stress of pollution. There is a need for preventive measures to be taken in order to avoid the indiscriminate direct discharge of this drug into nearby streams and ponds.

From the present investigation it observed that the lethal concentration (4.7g/lt) of oral contraceptives (Mala-D) has uniform arrangement of melanophores controlled scales; whereas dispersive action on melanophores and disturbing in their structure at early hours of exposure. In fishes and frogs, melanosomes dispersion is induced by elevation of intracellular cAMP levels, while aggregation is triggered by depression of cAMP (Reiter, 1985). Some other reports are also available on the effects of drugs or chemical compound on zebrafish embryos (Holmberg et al., 2006). Hence, it is concluded that the lethal concentration (4.7g/lt) of the contraceptives affect the external surface area and also nervous systems, thus melanophores indicate the stress of pollution. The random dispersion and structural alterations in melanophores in the exposed fishes are indicative of stress mediated production. The results clearly indicate that the addition of contraceptives to the water body may be a threat to aquatic fauna and flora.

Ethical Approval

The authors don't declare ethical approval.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Funding Statement

The authors don't declare any fund.

References

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

Ankley, G., Brooks, B., Huggett, D., & Sumpter, J. (2007). Repeating history: Pharmaceuticals in the environment. *Environment Science and Technolog,* 41, 8211-8217.

Bagnara, J.T., & Hadley, M.E. (1973). *Chromatophores and colour change the comparative physiology of animal pigmentation*. Prentice Hall, Inc, Englewood Cliffs, New Jersey. 1-191.

Fent, K., Weston, A.A., & Caminada, D. (2006). Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology*, 76, 122-159.

Fujii, R. (2000). The regulation of motile activity in fish chromatophores. *Pigment Cell Research*, 13, 300-319.

Fujii, R., & Oshima, N. (1994). *Factors influencing motile activities of fish chromatophores*. In: Advances In Comparative and Environmental Physiology (ed. R. Gilles) Springer-Verlag, Berlin, 20:1-54.

Holmberg, K., Bjerg, P.L., & Christensen, T.H. (2006). Occurrence and dis- tribution of pharmaceutical organic compounds in the groundwater downgradient of a landfill (Grindsted, Denmark). *Environmental Science and Technology*, 5, 1415-1420.

Larsson, D.G.J., Pedro, C., & Paxeus, N. (2007). Effluent from drug manufactures contains extremely high levels of pharmaceuticals. Journal of hazardous materials, 148, 751-755.

Miyashita Y., & Fujii R. (1973). Responses of guppy melanophores to 5-Hydroxytryptamine. *Journal of Pre-Medical course Sappro Medical,* Cll, 14, 39-44.

Parkar G.H. (1948). Animal control and their Neurohunorus. Cambridge press, Cambridge U.K. Scott GT 1965 Physiology and pharmacology of colour change in the sand flounder Scopthalamusaguosus. *Limnology and Oceanography*,10, R 2 30-R2 46.

Praveen, S., Gaur, A., & Ovais, M. (1993). Effects of potassium and alkaline earth ions on the isolated scale melanophores of a major carp *Cirrhinusmrigala*(Ham). *Journal Fresh Water Biology*, 5 (3), 277-282.

Rehman, M.S.U., Rashid, M., Ashfaq, M., Saif, A., Ahmad, N., & Han, J.I. (2015). Global risk of pharmaceutical contamination from highly populated developing countries. *Chemosphere*. 138, 1045-55. DOI: 10.1016/j.Chemosphere.201.02.036.

Saravanan, M. (2013). Toxicological effects of the antibiotics oxytetracyclin to an Indian major carp *Labeo rohita. Archives Environmental Contamination and Toxicology*, 64 (3), 494-503

Saravanan, M., Karthika, S., Malarvizhi, A., & 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 Material*, 195, 188-194.

Scott, G.T. (1965). Physiology and pharmacology of colour change in the sand flounder *Scopthalamus aguosus*. *Limnology and Oceanography*, 10, R2 30-R2 46.

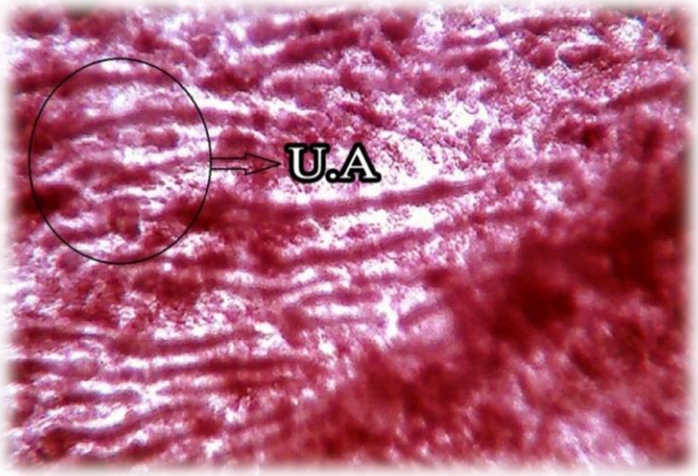
Sinha, M.K., Sinha, A.K., & Soma, A. (1999). Vertebrate colour change. *Biological Reviews*, 17, 120-150.

Watanable, M., Naitoh, T., & Suchya, K.T. (1965). Reversal of action of electrolytes to the melanophores of Cucian carp. *Biology Journal, Okayama University*, 11, 19-30.

Yamada, K.I., Miyata, S. & Katayama, H. (1984). Auto radiographic demonstration of adrenergic inervation to scale melanophores of a teleost fish. *Journal Experimental Zoology*, 22 (9), 78-80.

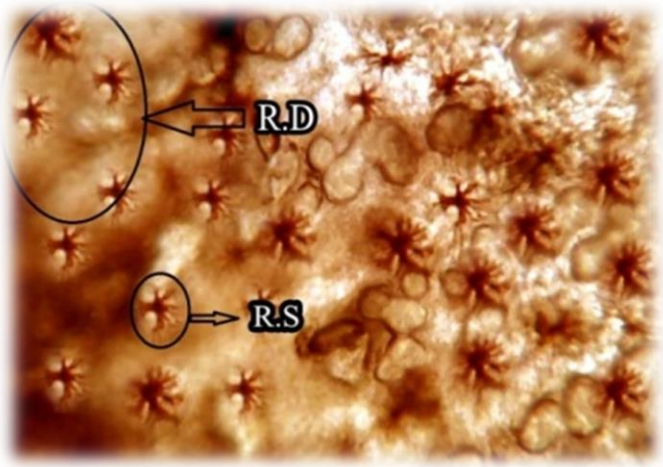
Zhang, Y., Geisen, S.U., & Cal, C. (2008). Carbamazepine and diclofenac: Removal in wastewater treatment plants and occurrence in water bodies. *Chemosphere*, 73, 1151-1161.

Plate-01: The population of normal chromatophore in the lateral region of fresh water fish, *Catla catla*

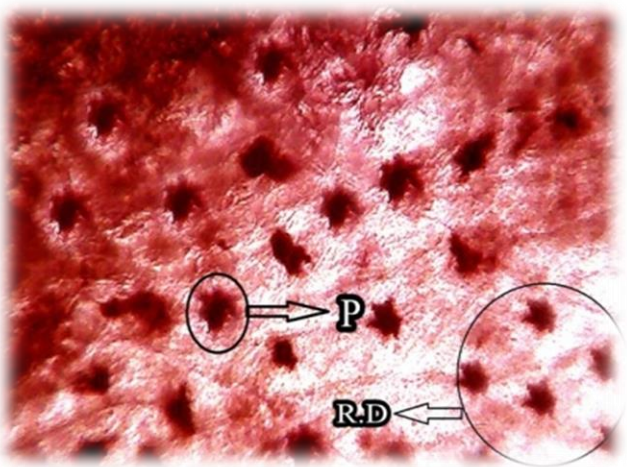


Catlacatla (low power) U.A = Uniform arrangement

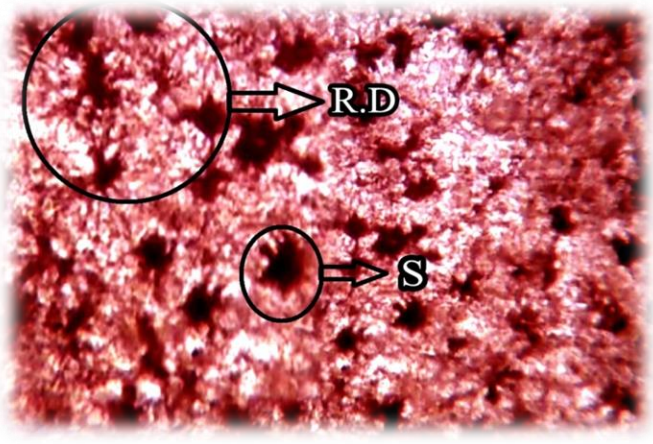
The alteration anddifferent types of the chromatophore at lateral region of the fresh water fish *Catlacatla*exposed to lethal concentration of oral contraceptive pills (MALA D) (4.7grms/ltr) at different hours. D = Random Dispersal, R.S = Reticulo Stellate, P = Punctate, S = Stellate



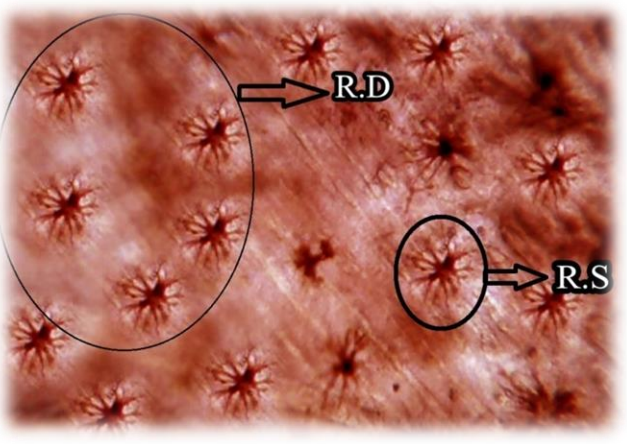
24 hours (10 x)



48 hours (10 x)

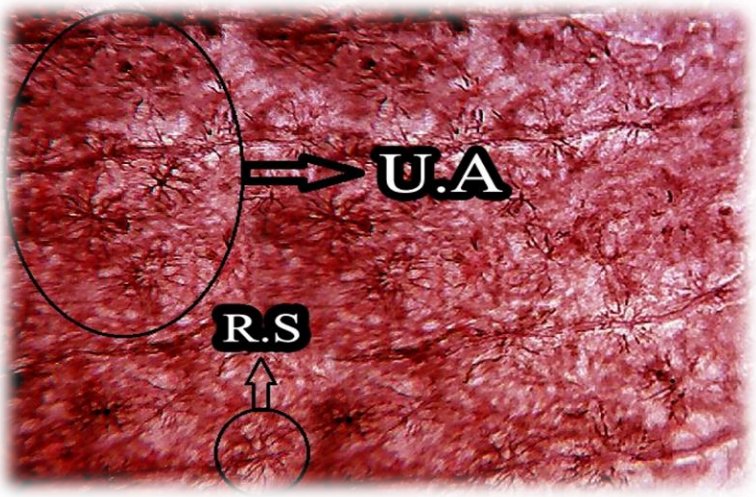


72 hours (10 x)



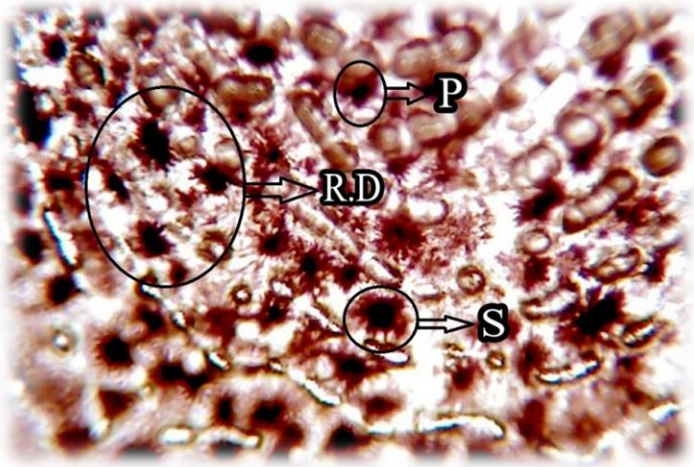
96 hours (10 x)

Plate-02: The population of normal chromatophore in the lateral region of freshwater fish *Cyprinus carpio*

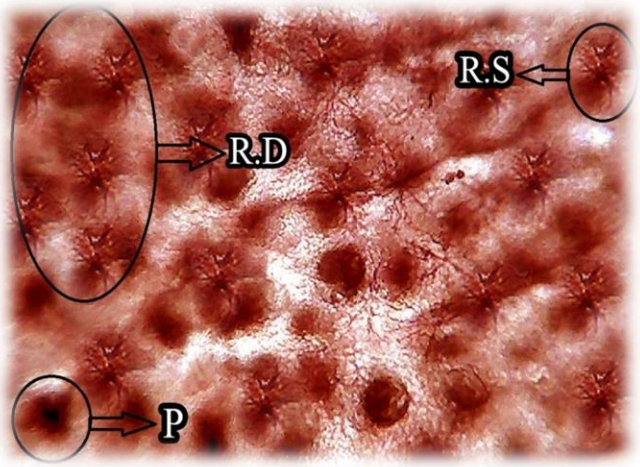


Catlacatla (low power) U.A = Uniform arrangement

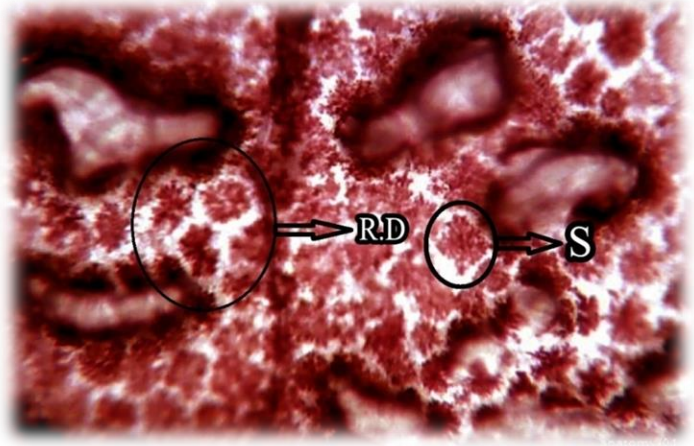
The alteration and different types of the chromatophore at lateral region of the Freshwater fish *Cyprinus carpio* exposed to lethal concentration of oral contraceptive pills (MALA-D) (4.7grms/ltr) at different hours D = Random Dispersal, R.S = Reticulo Stellate, P = Punctate, S = Stellate



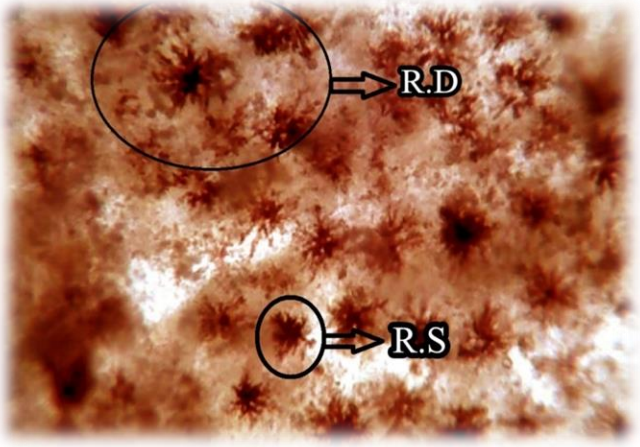
24 hours (10 x)



48 hours (10 x)

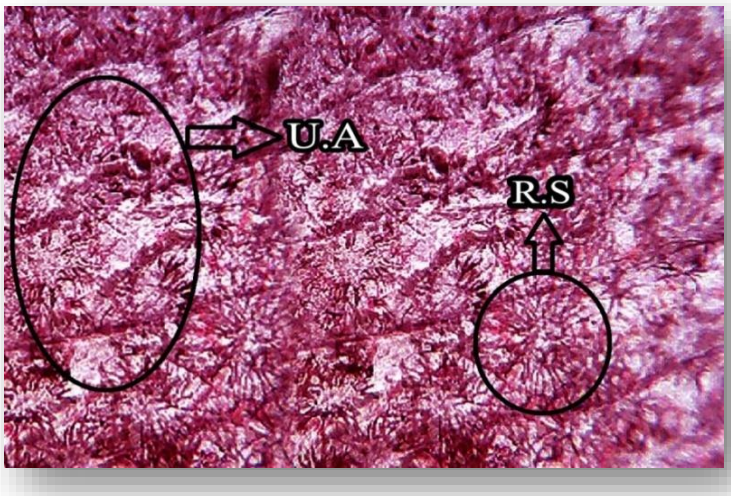


72 hours (10 x)



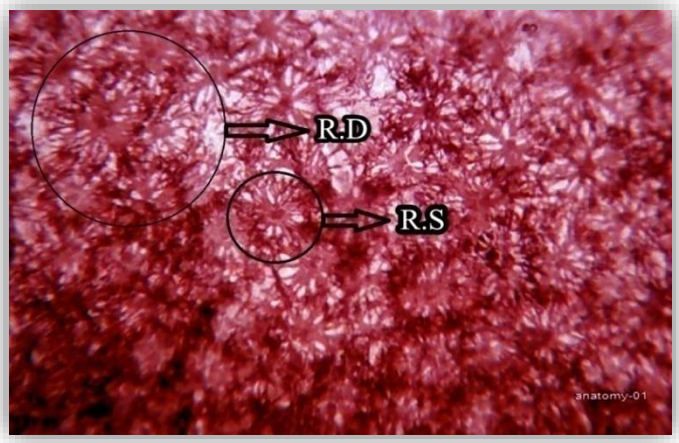
96 hours (10 x)

Plate-03: The population of normal chromatophore in the lateral region of freshwater fish *Labeo rohita*

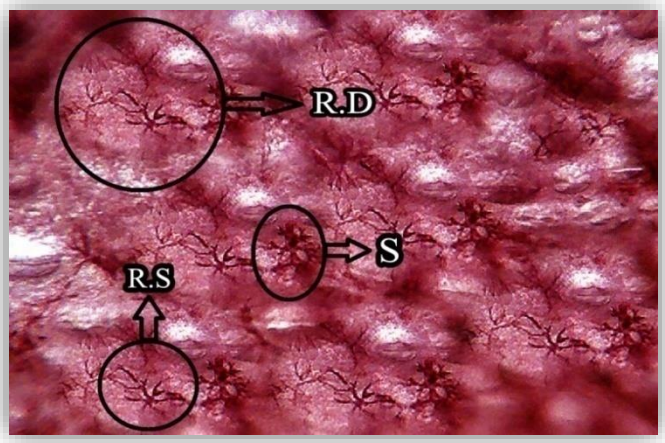


Labeo rohita (low power) U.A = Uniform arrangement

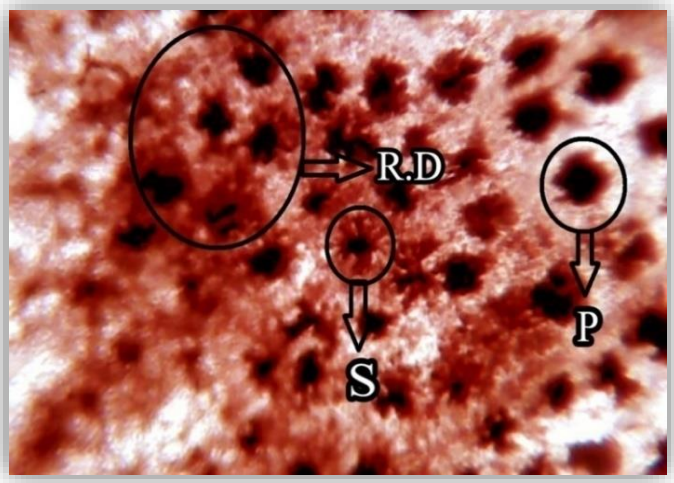
The alteration and different types of the chromatophore at lateral region of the freshwater fish *Labeo rohita* exposed to lethal concentration of oral -D) (4.7grms/ltr) at different hoursD = Random Dispersal, R.S = Reticulo Stellate, P = Punctate, S = Stellate



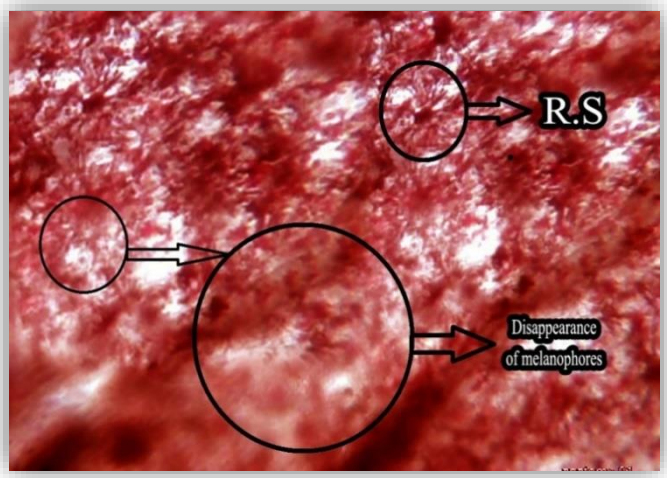
24 hours (10 x)



48 hours (10 x)



72 hours (10 x)



96 hours (10 x)