Int J Pharm Bio Sci 2013 July; 4(3): (P) 721 - 729

Research Article **Metal Toxicity**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

CADMIUM INDUCED OXDATIVE STRESS IN HEPTAOCYTES OF SNAKEHEAD FISH CHANNA PUNCTATUS (BLOCH.)- A INVITRO STUDY

K.K. SHUKLA*, DIWAKAR RAM TRIPATHI, ANURADHA SHUKLA AND J.P.SHUKLA**

P.G. Deptt. Of Zoology,S.Kisan P.G. College, Basti (U.P.)
***A.N.D. Kisan P.G.College, Babhnan, Gonda (U.P.)*
**IITR (CSIR Laboratory), M.G. Marg, Lucknow (U.P.)INDIA*

ABSTRACT

Present study has been undertaken to assess the toxicity of cadmium in vitro for viability of hepatocytes, lactate dehydrogenase leakage, glutathione and lipid peroxidation in *Channa punctatus* (Bloch), a freshwater snake head fish. Three concentrations of cadmium as cadmium chloride (0.1µg/ml; 3.0µg/ml and 5.0µg/ml) besides one control has been selected. Observations reveal that except lowest concentration of cadmium (0.10µg/l), the rest two concentration (3.0µg/ml and 5. 0µg/ml) exerted significant decline ($P<0.05$) in the viability of heptocytes, lactic dehydrogenase leakage and glutathione as compared to control. However, significant increase ($P<0.05$) was noticed in the lipid peroxidation in terms of MDA (Malon-di aldehyde) treated with 3.0µg/ml and 5.0µg/ml of cadmium chloride concentration as compared to control. Lowest concentration (1. 0µg/ml) of cadmium chloride could produce no significant alteration in lipid per oxidation. Causo-mechanism of these findings has been discussed in this paper.

KEYWORDS: ***Channa punctatus,*** **Cadmium chloride, Hepatocytes, viability, Lactic dehydrogenase, Glutathione, Lipid peroxidation, Cytotoxicity.**

K.K. SHUKLA
IITR (CSIR Laboratory), M.G. Marg, Lucknow (U.P.)INDIA

Corresponding author

INTRODUCITON

Cadmium is a heavy metal environmental pollutant. Its hepatotoxic, nephrotoxic, neurotoxic, reproductive and respiratory effects in animals are well documented. It is known to be highly toxic even in small concentrations (Scot and Slowman,2004; Hattink *et al.,*2005; Keserwani *et al.,* 2009; Sringa *et al.,*2010). Cadmium has also been recognized as carcinogen through epidemiological study (Takenaka et al.,1983). This metal is also reported to increase the permeability of cell membrane and causes metabolic cellular impairment (Grose et al.,1987). Cadmium is a heavy metal present in the aquatic ecosystem due to the industrial discharges, mining activities and small-scale industries. As a non-degradable cumulative pollutant, Cadmium can alter aquatic trophodynamics for centuries. Freshwater fishes are particularly vulnerable to metal exposure (Sorenson, 1991). Because fishes are important food resources and major ecosystem component, it has always been considered of relevance to assess the physiological effect of heavy metals in the aquatic environment on their morbidity, mortality, behavior, reproductive performance and physiological effects on its vital body-organs. The adverse effects of cadmium on growth, reproduction and osmoregulation in fishs are well documented (Pratap and Wendelan 1990). The activity of metabolic enzymes in liver, kidney, muscles and other tissues are reported to be disturbed following cadmium exposure (Sastri and Subhadra 1982). Although adverse effect of cadmium on various physiological function of fishes are well documented, still the mechanism of these effects sparsely worked out.

Though, literatures are available on the toxicity of various metallic pollutants and industrial wastes on many biochemical and histopathological parameters of fishes (Shukla et al.,2008; 2009; 2011a,b; Shukla and Shukla 2012a,b,c,d and e), however, sporadic studies have been conducted on the mechanism of cytotoxicity due to various heavy metals in general and cadmium in particular. Keeping this in view, the present study has been undertaken. Isolated mammalian hepatocytes have been proved to be an useful model for studying the mechanism of action of chemical toxins (Rauckman and Padilla 1987). Cadmium has been reported to induce heptotoxicity in vivo and monolayer of non-proliferating heptocytes has been established to be one of the best suited model for the toxicological studies because hepatocytes in cultures are representative of the response of differentiated liver cells in vivo (Rauckman and Padilla, 1987). The environmental pollutants may cause oxidative stress in aquatic organism by catalyzing conversion of oxygen to dangerous oxygen species (DOS) such as super oxide anions (O^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-). These DOS could damage macromolecules of cells. The intracellular antioxidants such as super oxide dismutase, catalase and reduced glutathione protects the cells from oxidative damage caused by these proxidants. The imbalance caused by the xenobiotics between pro-oxidants and antioxidants in favor of the former leads to oxidative damage of the cells. Two established parameters for oxidative stress are lipid peroxidation and reduced glutathione. The cytotoxicity can be determined by enzyme leakage and loss in viability. The purpose of this study is to evaluate the insult of oxidative stress to primary culture of fish hepatocytes which can provide a greater insight into the mechanistic study of cytotoxicity due to cadmium.

MATERIALS AND METHODS

Chemicals and reagents

Dulbacco's Modified Eagle Medium (DMEM), Fetal Calf Serum (FCS), Hanks Balanced Salt Solution (HBSS) were procured from Himedia laboratory Pvt. Ltd., Mumbai, India. Collagenase were purchased from Sigma, St. Louis, MO. Pencillin, streptomycin and Amphotericine-B

were also purchased from Himedia, Mumbai, India. Tissue culture dishes were obtained from Tarson, India).

Cell culture

The fish, *Channa punctatus* (Bl.) weighing 250±8.24 gm. were procured from local lake and acclimatized in laboratory conditions for 10 days. Hepatocytes were isolated according to the method of Birnbaum *et al*.,(1976). Briefly, fish was incapacitated by giving a blow to the head, an incision was made on the ventral side starting from anal region to the gill region so as to expose the abdominal cavity. Liver was removed aseptically and placed in calcium free buffer for 25 minutes. Thereafter, liver was transferred to a petridish containing Dulbacco's modified eagle medium (DMEM) containing collagenase (0.5%) and antibiotics (Penicillin 100/µm/ml,streptomycin 75µg/ml and amphotericine-B 2.5µg/ml), chopped in small pieces and incubated for one hour with manual shaking. Thereafter, the cells were filtered with 100µ nylon mesh. Filtrates containing freed cells were centrifuged at 1000rpm for 10 minutes. Pellet was resuspended in fresh medium and again centrifuged. This process of washing of hepatocytes by centrifugation was repeated for five times. After final washing pellet was resuspended in medium FCS, diluted with DMEM and cell yield and viability was counted on haemocytometer. Now cells, at the density of $1x10^6$ cells/ml were seeded in 35mm culture dish. All the above processes were carried out in the laminar flow. The cell in the petridishes were incubated in humidified atmosphere at 5% CO_2 and 27 ^0C temperature to facilitate monolayer formation for 24 hours.

Treatment

After 24 hours of incubation, medium from each dish was replaced with test medium containing different concentrations of cadmium (0.0,1.0,3.0, and 5.0µg/ml as cadmium chloride) and incubated for 2 hours. The experiment was conducted in triplicate. After treatment period happened over, medium from each petridish was collected and kept for determination of lactate dehydrogenase activity. Monolayer of hepatocytes was washed with PBS and cells were collected in 2ml phosphate buffer by aspiration.

Viability Assay

Viability of detached hepatocytes wre examined by trypan blue dye exclusion test (Nicotera and Orenius, 1994). Monolayer of hepatocytes was washed with PBS, cells were pelleted, resuspended and mixed with 0.4% trypan blue (1:1 ratio) and viable cells were counted on haemocytometer.

Lactate dehydrogenase estimation

After 2 hours of exposure period, medium from both control and experimental dishes was collected and centrifuged at 1000 rpm for 5 minutes. Pellet was discarded and LDH leakage in medium was examined according to the method of Kornberg (1955).

Glutathione estimation

Reduced glutathione (GSH) in hepatocytes were estimated according to method of Richardson and Murphy (1975). Briefly, culture plates were washed with PBS, cells were collected in phosphate buffer and lysed by repeated freezing and thawing of cells and centrifuged at 1000rpm for 10 minutes. In 0.5ml of supernatant was added 0.5ml of 10% TCA and centrifuged for 10 minutes. Now in 1.0ml of supernatant was added 1.0ml of phosphate buffer and 1.0ml of DTNB (0.01N) and absorbence was read at 412nm

Quantification of lipid peroxidation

Lipid peroxidation in hepatocytes was examined according to method of Beuge and Aust (1969). Briefly, monolayer was washed with PBS and cells were collected in 2ml of phosphate buffer and were lysed by repeated freezing and thawing for five times. In 0.5ml aliquot of cells was added 1.2ml Trichloroacetic acid (TCA 15%w/v) 0.5ml, Tribarbituric acid (TBA 1%w/v pH 7.0-8.0) and 1.8ml water and were kept in boiling water bath for 30 minutes. Absorbence was read at 532nm.

RESULTS AND DISCUSSION

Effect on viability

Effect on the viability of the hepatocytes is shown in table 1. The lowest concentration of cadmium showed no significant loss in viability of the hepatocytes as compared to control. However, rest of the two concentration of cadmium showed significant loss in viability of the cells compared to control (Table 1 and fig 1).

Effect on Lactate dehydrogenase leakage

The effect of cadmium on the leakage of lactate dehydrogenase from the hepatocytes is shown in Table 2 & Fig.1. The highest concentration of cadmium exhibited significant leakage of lactate dehydrogenase in the medium compared to control and the rest of concerntraion of cadmium. However, the effect of lowest concentration of cadmium (1.0µg/ml) on leakage of LDH was not significant.

Effect on reduced glutathione

The lowest concentration of cadmium revealed no significant reduction in the level of reduced glutathione of hepatocytes as compared to control. However, rest of the concentration of cadmium showed significant reduction of glutathione as compared to control groups. (Table 3 and Fig.1).

Effect on lipid peroxidation

Table 4 and Fig.1. shows the result of MDA level from control as well as cadmium exposed hepatocyte culture. The level of MDA in cadmium exposed hepatocytes were significantly higher in all the concentration of cadmium except lowest cadmium concentration as compared to control. Isolated and cultured hepatocytes are being used for routine metabolism and toxicity testing as well as for investigation on the regulation of liver function because monolayers of non-proliferating hepatocytes exhibit characteristic function of liver in vivo (Rauchkman and Padilla, 1987). In the present study, decrease in the cell viability, leakage of LDH from the cells, reduction in Glutathione and increase in lipid per oxidation was observed in the cadmium treated hepatocytes of the fish. This decrease in viability may be due to the cytotoxicity to the cell when exposed when to varied concentration of cadmium. Concomitant dose-dependent changes in other parameters associated with oxidative stress may lead to damage to the macromolecules and cellular physiology which ultimately lead to cell death (Nicotera and Orrenius, 1994). In hepatocytes, the correlation between loss of cell viability and the release of LDH is well established (Moldeus *et al., 1979*). Cell membrane under normal physiological condition is nearly impermeable to macromolecules. A damage to the cell membrane leads to the leakage of intracellular enzymes into the extracellular space (Mol *et al.,* 1986). Lactate dehydrogenase leakage is an established parameter for evaluation of cytotoxicity caused by xenobiotics (Mitchell and Acosta, 1980). In the present study, cytotoxicity caused by the cadmium is evidenced by the leakage of LDH in medium. Similar findings were also reported by Mitchell and Acosta, 1980., Bartnic et al.,1990.

The cytotoxicity caused by xenobiotics may be due to the oxidative stress produced within the cell. The degree of oxidative stress can be determined by measuring the concentration of certain proteins, lipid peroxidation byproducts, MDA (malondialdehyde) and antioxidant enzymes which combine to mediate the intracellular response to increase oxidative stress (Ceruti *et al.,* 1988). Reduced glutathione (GSH); a non-protein thiol group protects the cell from oxidative stress by scavenging the free radicals and oxidative byproducts (Reed and Beaty, 1980). Cadmium exposure to the laboratory animals is reported to generate free radicals which cause oxidative stress (Hirano *et al.,* 1997, Abe *et al.,* 1998). Similarly, in isolated rat hepatocytes, cadmium is reported to generate oxidative stress as reported by Hussain *et al.,* (1987). Our findings are also in accordance with these observations where cadmium exposure to fish hepatocytes has been found to induce oxidative stress as revealed by reduced

glutathione levels in the exposed cells. The decrease in the glutathione may be due to one of the following reasons : (1) the activity of enzyme y-glutamyl transpeptidase is increased as suggested by Karmakar *et al.,* (1998); (2) the enzyme glutathione reductase is involved in conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) in presence of cofactor NADPH. Because of increased level of lipid peroxidation, this NADPH is less available to the enzyme and, therefore, conversion of GSSG to GSH does not occur as suggested by Sarkar *et al.,* (1995). Glutathione depletion due to exposure of cadmium decreases the GSH/GSSG ratio. Consequently, the production of free radicals exceeds the scavenging capacity of GSH (Leelank & Bansal, 1996). Lipid peroxidation is one of the best known manifestation of oxidative cell damage. Olivares *et al.,* (1997) reported marked reduction of glutathione level and increased lipid peroxidation due to ethanol and acetaldehyde in rat hepatocytes. In this study we observed a significant increase in the lipid peroxidation following cadmium exposure. This increase in lipid peroxidation may be due to the decrease in the level of glutathione. The interaction of prooxidants with membrane lipids results in to the production of malondialdehyde and the accumulation of this oxidation products is indication of enhanced oxidative stress (Belghmi *et al.,* 1988). Increase in lipid peroxidaton in a dose dependent manner suggests that GSH depletion may not be a prime reason for increased oxidative damaged products. In conclusion, it can be presumed that hepatocytes from fish *Channa punctatus* (Bloch) is sensitive to oxidative damage caused by the cadmium and can be used in assessing metal toxicity on the cells for screening and regulatory purposes.

Table 1

Percent viability of fish (Channa punctatus) hepatocytes exposed to various concentrations of cadmium in vitro

Concentration of Cd (µg/ml)	Percent viability of cells
0.0	89.89±0.742
1.0µg/ml	86.81±1.99
3.0µg/ml	83.12±2.06*a,b
5.0µg/ml	75.79±0.641*a,b,c

Values are mean ± S.E. of three culture dishes per treatment.
Significance * p<0.05 statistical evaluation by one way ANOVA
A-Vs Control
B-Vs 1.0 µg/ml
C-Vs3.0µg/ml

Table 2

LDH leakage from fish (Channa punctatus) hepatocytes exposed to various concentrations of cadmium in vitro

Treatment group (Cd µg/ml)	LDH leakage nm/min. /mg protein
0.0	16.57±0.613
1.0µg/ml	19.92±1.4
3.0µg/ml	20.30±1.83*
5.0µg/ml	37.03±6.24*a,b,c

Values are mean ± S.E. of three culture dishes per treatment.
Significance * p<0.05 statistical evaluation by one way ANOVA
A-Vs Control
B-Vs 1.0 µg/ml
C-Vs 3.0 µg/ml

Table 3

Effect of cadium on reduced glutathione levels in primary culture of fish (Channa punctatus) hepatocytes in vitro

Treatment group (Cd µg/ml)	Glutathione level nm/mg protein
0.0	32.34±2.98
1.0µg/ml	28.50±3.75
3.0µg/ml	22.06±1.68*a,b
5.0µg/ml	18.93±0.59*a,b,c

Values are mean ± S.E. of three culture dishes per treatment.
*Significance * p<0.05 statistical evaluation by one way ANOVA*
a-Vs Control
b-Vs 1.0 µg/ml
c-Vs 3.0 µg/ml

Table 4

Effect of cadmium on lipid peroxidation in primary culture of fish (Channa punctatus) hepatocytes in vitro.

Treatment group (Cd µg/ml)	MDA level nm/mg protein
0.0	3.31 ± 0.293
1.0µg/ml	2.88 ± 0.115
3.0µg/ml	6.17 ±0.375*a,b
5.0µg/ml	14.13±1.35*a,b,c

Values are mean ± S.E. of three culture dishes per treatment.
*Significance * p<0.05 statistical evaluation by one way ANOVA*
- Vs Control
B-Vs 1.0 µg/ml
C-Vs 3.0 µg/ml
D-MDA = Malon Di Aldehyde

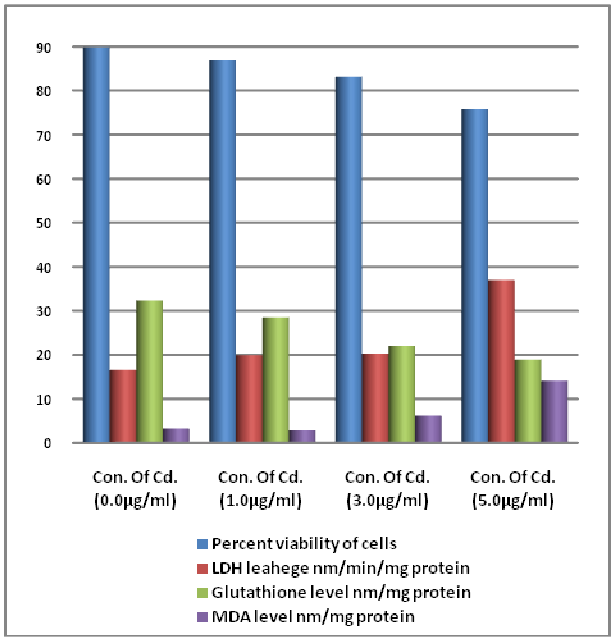

Figure 1

Showing percent viability of hepatocytes, LDH lekage, reduced glutathione and MDA level exposed to 1.0, 3.0 and 5.0µg/ml in (Channa punctatus) when compared with control.

ACKNOWLEDGEMENT

One of us (JPS) thanks to UGC, New Delhi for providing major research project No. F.No. 38-183/2009 (SR) dt Dec. 24. 2009. Authors also thank to Prof. P.K.Seth, Ex. Director IITR (CSIR Laboratory) Lucknow, U.P. for his deep involvement in conduction of this work and to Miss Neeta Adhikari for her cooperations in many ways.

REFERENCES

1. Abe.T, Yamamura. K, Gotoh.S, Kashimura. M.Higashi. K. (1998). Concentration dependent differential effect of N-acetyl-L-cysteine on the expression of HSP 70 and metallothionen genes induced by cadmium in humaniotic cells. Biochem. Biophys. Acta. 1380, 123-132.
2. Binbaum. M.J.,Schultz.J, Fain. J.N. (1976). Hormone-stimulatedglycogenolysi, in isolated gold fish hepatocytes. Am.J.Physiol, 231,191-197.
3. Buege.J.A and S.D.Aust (1978). Lipid peroxidation.In; Methods ir Enzymology. 52, pp 302.
4. Belghami.K, Nicolas.J.C, Crastes de Pault.A (1988). Chemiluminiscent assay of lipid hydroperoxides.J.biolumin. Chemukumin. 2, 113-119.
5. Bartnik.F.G,Pitterman.W.F., Mendorf.N, Tillman.U, and kunstler.K (1990). Skin organ culturesfor the study of skin irritancy. Toxicol. In. vitro, 4,293-301.
6. Bagchi.D, Bagchi.M, Hassonan. E.A, Sloliss.J (1996). Cadmium induced excertion of urinary lipid metabolites,DNA damage, GSH depletion and hepatic lipid peroxidation in sprague dawley rat. Biol.Trace. Elemen. Res 52, 143-154.
7. Ceruti. P.A., Krupitza.G, Larsson.R, Muchlematter.D, Cransford.D and Amstad.P (1988). Physiological and pathological effect of oxidants in mouse epidermal cells. Ann.NY.Acad.Sci 551,75-82.
8. Grose. E.C, Richards.J.H., Jaskot.R.H., Menache.M.G, Graham.J.A, Dauterman.W.C. (1987).A comparative study of effect of cadmium chloride and cadmium oxide; Pulmonary research. J. Toxicol. Environ. Health. 21, 219-232.
9. Hatinik, J.; D.B. Broeck and R. Blust (2005): The toxicokinetics of cadmium in carp under normoxic and hypoxic condition. Aquatic Toxicology. 75(1): 1-15.
10. Hirano.T, Yamaguchi.Y, Kasai.H (1997). Inhibition of β-hydrokiguanin repair in testes after administration cadmium chloride to glutathio deplected rat. Toxicol.Appl pharmacol. 147, 9-14.
11. Hussain,T, Shukla.G.S., Chandra.S.V. (1987). Effect of cadmium on SODand lipid peroxidation in liver and kidney in growing rats: in vivo and in vitro study. Pharmacol. Toxicol 60, 355-358.
12. Karmakar.K, Banik.S, Bandopadhyay.S, Chattergy.M (1998). Cadmium induced alteration of hepatic lipid peroxidation, glutathione level and possible correlation with chromosome aberration in mice: a time course study. Mutation. Research 397, 183-190.
13. Keserwani, D.; H.C. Lodhi; K.J. Tiwari; Sanjiv Shukla and U.D. Sharma (2009): Cadmium toxicity in freshwater Catfish, Heteropneustes fossilis (Bl.). Asian J. Exp. Sci., 23(1): 149-156.
14. Kornberg.A (1955). Lactate dehydrogenase in muscles. In:Clowick.S.P., Kaplan.N.O. (EDS)., Methods in Enzymology, voli, Acad press, NY, pp 441-443.
15. Leelank.B.N., Bansal. M.P.(1996). Effect of Se supplementation on glutathione redox system in the kidney of mice after chronic cadmium exposure. J.Appl. Toxicol 17, 81-84.

16. Mitchell D.B., Santone.K.S., Acosta.D (1980). Evaluation of cytotoxicity in cultured cells by enzyme leakage. J.Tissue.Culture. Method 6, 113-116.
17. Moldeus.P, Hogberg.J, Orrenius.S (1979). Isolation and use of liver cells. In; S.Fleischer and L.Packer (eds). Methods in Enzymology, vol II, part C, Acad. Press, NY, pp60.
18. Muller.L(1984). Differential sensitivity of integrity criteria as indicators of cadmium induces cell damage. Toxicol. Letter 21, 21-27.
19. Mol. M.A.E, Van-Genderaan.J, Woltherus.O.L. (1986). Cultured human epidermual cells as a tool in kin toxicology. Food. Chem. Toxicol 24, 519-520.
20. Nictoera.P, Orrenius.S (1994). Molecular mechanism of toxic cell death; an overvies,In:Tysan.C.A.,Frazier.J.M(eds, in vitro toxicity indicators. Academic Press. USA. Pp 23-32.
21. Olivares.I.P., Bucio.L, Sonca.V, carabaez.A, utieneruiz.M.C (1997). Comparative study of damage produced by acute ethanol and acetaldehyde treatment in human fetal hepatic cell line. Toxicology 120, 133-144.
22. Pratap.H.B, Wendelan Bonga. S.E.(1990). Effect of waterborn cadmium on plasama cartisol and glucoe in cichlid fish (Oreochromis osambicus).Comp. Biochem.physiol, 95c, 313-317.
23. Reed.D.J.Beatty.P.W. (1980). Biosysnthesis and regulationof glutathione in toxicology application. Biochem.Toxicol, 2, 213-241.
24. Rauckman. E.J, Padilla.G.M. (EDS) 1987).The isolated hepatocytes; Use in toxicology and xenobiotic biotransformation. Academic Press, NY, pp 292.
25. Sarkar.S, Yadav.P, Trivedi.R, Bansal.A.K, Bhatnagar.d(1995). Cadmium induced lipid peroxidation in and the status of antioxidant systems in rat tissue. J.Trace. Elemen. Med 9, 144-147.
26. Sastri. K.V, Subhadra.K (1982). Effect of cadmium on some aspect of carbohydrate metabolism in fresh water cat fish Heteropniustes fossilis. Toxicol. Letter 14, 45-51.
27. Stacey.N.H, Kappus.H (1982). Comparison of method of assessment of metal induced lipid peroxidation in isolated rat hepatocytes. O. Toxicol. Environ. Health. 9, 277-285.
28. Scott, G.R. and K.A. Sloman (2004): The effect of environmental pollutants on complex fish behavior: integrating behavioural and physio;ogical indicators of toxicity. Aquatic Toxicology. 68(4): 369-392.
29. Sorenson,E.M(1991). Cadmium, In Metal poisoning in fish. CRC, press, Boca Ratron; Florida, pp 175-234.
30. Shukla Anjani Kumar, Anuradha Shukla, J.PShukla & S.Shanker (2008): Chromium induced alternation in some biochemical profiles during testicular cycle of *Colisa fasciatus* (Vl.Schn.). J.Nat.Res. and Dev. 3(2):78-80.
31. Shukla Anuradha, J.PShukla (2009): Paper and pulp mill inducedalterationin glycogen and lipid during ovarian cycle of *Colisa fasciatus* (Bl.Schn.). a tropical freshwater perch. J.Nat.Res. and Dev. 4(2):59-63.
32. Shukla Anuradha, J.PShukla, Anjani kumar Shukla and M.Mishra (2011a):Hexavalent chromium induced nucleic acids and protein alteration in the testicular cycle of *Colisa fasciatus* (Bl.Schn); J.Adv.Zool. 32(2) 126-131.
33. Shukla Anuradha, J.PShukla, Anjani kumar Shukla (2011b): Nucleic Acids and Protein alteration in the ovarian cycle of *Colisa fasciatus* (Bl.Schn), under hexavalent chromium stress. Life Science Bulletin. 8(1): 87-90.
34. Shukla Anuradha, J.PShukla (2012a): Distillery effluent induced alteration in the nucleic acid and protein during testicular cycle of *Colisa fasciatus* (Bl.Schn), atropical fresh water perch. Int. J.Pha. & Bio. Sci. 3(1) 532-537.
35. Shukla Anuradha, J.P Shukla(2012b): Quantitative alteration in the nucleic acid and protein during ovarian cycle of *Colisa*

fasciatus (Bl.Schn), a tropical freshwater perch, under distillery effluent stress. . Int. J.Pha. & Bio. Sci. 3(3) 147-154.

36. Shukla J.P., Anuradha Shukla and Rakesh K.Dubey (2012c): Deleterious effects of hexavalent chromium on the blood pyruvate level in the fingerlings of *Channa punctatus* (Bl.), a tropical freshwater murrel. . Int. J.Pha. & Bio. Sci. 3(4) 789-794.
37. Shukla Anuradha, J.PShukla (2012d): Distillery effluent induced alteration in the hematological profile of fingerlings of *Colisa fasciatus* (Bl.Schn), a tropical freshwater perch. J.Env.Biol. (in press).
38. Shukla Anuradha, J.P.Shukla (2012e): Testicular cycle of *Colisa fasciatus* (Bl.Schn) under hexavalent chromium stress. Brithish J Phram. & Tax. (Maxwell Scientific Publication) (In press).
39. Srianga,T.P.; M.P. Mohapatra; G.K. Panigrehy; B.C. Guru and S.C. Patnaik (2010): Studies of effect of cadmium toxicity on protein metabolism in brain and muscle tissues of a freshwater teleost, *Channa punctatus.* The Ecoscan. 4(2 & 3): 189-192.
40. Stacey.N.H, Cantellena.L.R., Klaassen.C.D. (1980). Cadmium toxicity and lipid peroxidation in isolated rat hepatocytes. Toxicol. Appl. Pharmacol 53, 470-480.
41. Takenaka.S, Oldieger.H, Konig.H, Hochrainer.D, Oberdorster.O (1983). Carcenogenicity of cadmium chloride, aerosol in winstar rats J.Natl.Cancer. Inst 70, 367-373.